ISSN 0037-9832

VOL 25 NO 1 JANUARY 1974

Journal of the Society of Cosmetic Chemists

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Biochemistry of the Acute Allergic Reactions

Edited by K. Frank Austen and Elmer L. Becker. 1972. 368 pages, 96 illustrations. £4.00.

The published record of this symposium serves not only as a more or less complete summary of our present knowledge of the subject but also as a comparison with the published proceedings of the First International Symposium to indicate the nature of the progress that has occurred. The progress is a steady one in all aspects of the field touched upon. Particularly striking is the advance made in studying the genetic control of homocytotrop antibody production, the demonstration of the involvement of cyclic AMP in various allergic cellular and tissue responses, the isolation in pure or almost pure form of the various components of the kinin-forming system, and the recognition of the alternate or bypass system of complement activation. This is an absolutely outstanding symposium in terms of the participants and the originality and importance of their contributions. It is timely, and there is every reason to believe that the demand for this volume will greatly exceed that for the first symposium.

Textbook of Dermatology

Edited by Arthur Rook M.D. F.R.C.P., D. S. Wilkinson M.D. F.R.C.P. and F. J. G. Ebling D.SC. PH.D. *Second Edition*, 1972. 2236 pages, 1100 illustrations. Two volumes in slip case, £32.50.

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Allergic Diseases: Diagnosis and Management
Edited by Roy Patterson M.D. 1972. 672 pages, 74 illustrations (8 colour). Lippincott, £11.90

This book covers those clinical problems that are commonly seen in the daily practice of allergy. It is a modern and up-to-date reference work written by eighteen specialists whose wide experience and authority ensure that the book will become recognized as an important addition to the literature on the diagnosis and treatment of allergic diseases. It will be useful to all physicians who are occasionally confronted with allergic problems in their practice, as well as specialists in allergy and immunology.

Manual of Skin Diseases
Gordon C. Sauer M.D. *Third Edition*, 1973. 442 pages, 589 illustrations (237 colour). Lippincott, £12.00

Nearly 200 new illustrations are among the changes and additions made to the third edition of this well-known manual. There are also new chapters on paediatric dermatology, geriatric dermatology, photosensitivity, skin problems and hereditary skin problems. This book is a refreshing change from the weighty tomes of the standard texts and its wealth of illustrations present virtually all known skin diseases with the utmost clarity. This is an essential aid which should be available to everyone working in dermatology and is likely to be in constant use as a library and laboratory reference.

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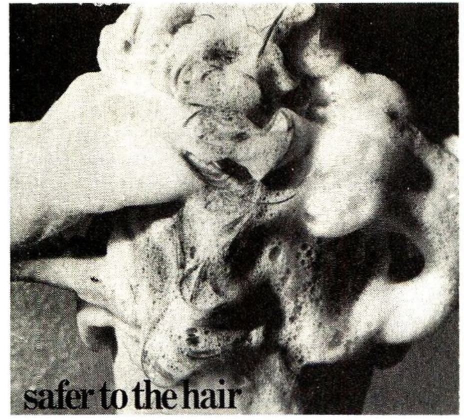

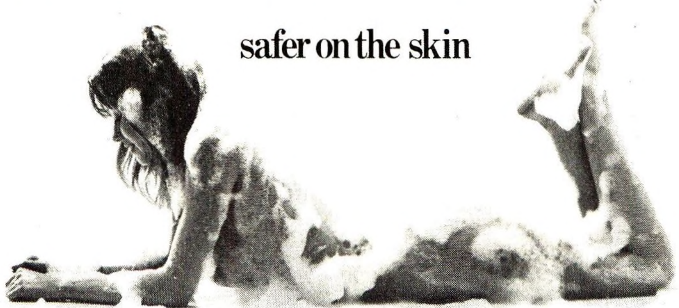

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127×76 mm index cards for reference without mutilating the pages of the Journal.

The stability of the monofluorophosphate and fluoride ions in dentifrice containing calcium carbonate: BENGT NORÉN and CATRIN HÄRSE. *Journal of the Society of Cosmetic Chemists* **25** 3–11 (1974)

Synopsis—The stability of sodium monofluorophosphate and sodium fluoride in dentifrices with calcium carbonate as the only polishing agent has been investigated by a one year storage test. The ratio of the fluorine components was varied, whereas the total amount was constant.

It was found that sodium fluoride was not completely inactivated by the calcium carbonate dentifrice. The inactivation due to formation of insoluble calcium fluoride within a few months reached an equilibrium which had a high content of free fluoride ions—about 40% of the added amount.

When sodium monofluorophosphate was present it dissociated and liberated fluoride ions. Depending on the initial ratio of monofluorophosphate to fluoride in the dentifrice the free fluoride content either increased or at first decreased, passed a minimum and then increased. It was also possible to balance the inactivation of fluoride ions by the contribution of fluoride ions from the dissociation of monofluorophosphate to get a constant free fluoride ion content.

The chemistry of human hair cuticle—I: A new method for the physical isolation of cuticle: J. A. SWIFT and B. BEWS. *Journal of the Society of Cosmetic Chemists* **25** 13–22 (1974)

Synopsis—The cuticle of human hair has been isolated in bulk by a new method involving vigorous agitation of fibres in water. The cuticle fractions have been shown to be of high morphological purity using various techniques of electron microscopy. The significance of amino acid analyses is discussed.

The substantivity of cosmetic ingredients to the skin, hair and teeth: N. J. VAN ABBÉ. *Journal of the Society of Cosmetic Chemists* **25** 23–32 (1974)

Synopsis—Substantivity conveys the idea of prolonged association between a material and a substrate, an association which is greater or more prolonged than would be expected with simple mechanical deposition. This review is intended to discuss the advantages and disdvantages of substantive effects, various ways of achieving substantivity and methods for its detection and assessment.

The following papers have been accepted for publication in the *Journal*:

ORIGINAL SCIENTIFIC PAPERS

The behaviour of perfumery ingredients in products
J. W. K. Burrell, Ph.D., B.Sc., A.R.C.S., D.I.C.

Decision analysis and its relevance to subjective testing
A. H. Christer, B.Sc., M.Sc., Ph.D., A.F.I.M.A.

Response of the frog olfactory system to controlled odour stimuli
T. M. Poynder, B.Sc., A.R.I.C.

SUBJECT REVIEW ARTICLES

Sesquiterpenes in the perfumery industry
H. R. Ansari, M.Sc., Ph.D., A.R.I.C. and A. J. Curtis, B.Sc., A.R.I.C.

Specialized techniques for the analysis of cosmetics and toiletries: D. M. Gabriel. *Journal of the Society of Cosmetic Chemists* **25** 33–48 (1974)

Synopsis—It is only during the last century that scientific principles have been applied to the development of cosmetics and toiletries, but during the same span of time there have been dramatic advances in analytical techniques, many of which have been applied to solve particular analytical problems associated with this industry.

Modern analysis is generally a two stage procedure—first the separation and isolation of the various components of interest followed by characterization, identification and estimation.

Examples illustrate the use of a wide range of techniques which have been applied to the analysis of shampoos, aerosol hairsprays, hairdressings, toothpastes, antiperspirant/deodorants and talcum powders.

A suggested reading list is appended.

The application of microcalorimetry to research in the field of toilet preparations: G. P. Adams. *Journal of the Soceity of Cosmetic Chemists* **25** 49–58 (1974)

Synopsis—A differential microcalorimeter has been adapted for adsorption studies on biological substrates. The instrument provides reproducible quantitative information on the heat change associated with the interaction of the material of interest with the substrate. When this heat change is combined with information on the amount of material adsorbed obtained using conventional analytical techniques, an indication of the adsorbate-adsorbent interaction is obtained. The calorimeter is also capable of giving information on the rate of adsorption, the time required to reach thermal equilibrium and whether or not appreciable adsorption occurs in times encountered during product application. Subsidiary experiments indicate whether the adsorbed material is likely to withstand rinsing or whether the adsorption characteristics are grossly affected by the presence of detergent.

The paper describes the apparatus and techniques employed and gives general examples of the applications of the method to illustrate its utility.

Journal of the Society of Cosmetic Chemists

This edition is published for

THE SOCIETY OF COSMETIC CHEMISTS
OF GREAT BRITAIN

by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL

Hon. Editor: J. M. Blakeway

Chesebrough Pond's Ltd, Victoria Road, London NW10 6NA

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VOL. 25 JANUARY 1974 No. 1

GENERAL NOTICES

Publication dates: The 'Journal of the Society of Cosmetic Chemists' is published on the 5th of each month.
Five issues for the Society of Cosmetic Chemists of Great Britain
56 Kingsway London WC2B 6DX.
Seven issues by the Society of Cosmetic Chemists
50 East 41 Street, New York, N.Y. 10017, U.S.A.

Issue No	*Publication Date*	*Country of Origin*
1	January	Great Britain
2	February	U.S.A.
3	March	U.S.A.
4	April	Great Britain
5	May	U.S.A.
6	June	Great Britain
7	July	Great Britain
8	August	U.S.A.
9	September	U.S.A.
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11	November	U.S.A.
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Advertisements: All enquiries regarding advertisements in the British Editions of the Journal should be addressed to Blackwell Scientific Publications, Osney Mead, Oxford OX2 0EL.

Subscription: All members of the Society of Cosmetic Chemists of Great Britain receive one copy of each edition free. Further copies at non-member rates. Industrial and non-member subscribers: £21. Non-profit institutional subscribers: £14 per annum, post free Single issues: £2.10. If payments are made by bank transfer, all charges shall be at the remitter's exsense.

Missing numbers: **Journals are despatched at Printed Paper rate. Claims for missing numbers can be entertained only from subscribers in the country of origin of the particular issue, and must be made within 30 days from date of issue. Members and subscribers are urged to give notice of change of address to the Publications Offices.**

Responsibility for statements published: The Society of Cosmetic Chemists of Great Britain and its Hon. Editor assume no responsibility for statements or opinions advanced by contributors to this Journal.

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Journal of the Society of Cosmetic Chemists

1974

VOL. 25

Journal of the Society of Cosmetic Chemists

VOL. 25 1974

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The stability of the monofluorophosphate and fluoride ions in dentifrice containing calcium carbonate

BENGT NORÉN and CATRIN HÄRSE*

Synopsis—The stability of sodium MONOFLUOROPHOSPHATE and sodium FLUORIDE in DENTIFRICES with CALCIUM CARBONATE as the only polishing agent has been investigated by a one year storage test. The ratio of the fluorine components was varied, whereas the total amount was constant.

It was found that sodium fluoride was not completely inactivated by the calcium carbonate dentifrice. The inactivation due to formation of insoluble CALCIUM FLUORIDE within a few months reached an equilibrium which had a high content of free fluoride ions—about 40% of the added amount.

When sodium monofluorophosphate was present it dissociated and liberated fluoride ions. Depending on the initial ratio of monofluorophosphate to fluoride in the dentifrice the free fluoride content either increased or at first decreased, passed a minimum and then increased. It was also possible to balance the inactivation of fluoride ions by the contribution of fluoride ions from the dissociation of monofluorophosphate to get a constant free fluoride ion content.

INTRODUCTION

Several investigators have shown that fluorine-containing dentifrices give a caries reduction in children. The fluorine component of the dentifrices has mainly been added in the form of stannous fluoride, sodium fluoride, sodium monofluorophosphate or amine fluoride.

Certain problems have arisen, however, as the fluorine components, especially the fluorides, were not compatible with the commonly used polishing agents. The uptake of fluorine compounds has been investigated by Ericsson (1) and Koch (2). However, only one fluorine concentration and a rather short contact time was studied.

* Research Department of the Kabi Group, S-104 25 Stockholm, Sweden.

Despite the predominant use of fluorine-containing dentifrices in Sweden, when this investigation was started nothing had been published on the stability of sodium monofluorophosphate or sodium fluoride in dentifrices with precipitated calcium carbonate as the only polishing agent. Recently, however, Andersson and Ericsson (3) published a study on fluorine components in the liquid phases of 7–8-years-old dentifrices and newly produced dentifrices (*Vademecum*®)* respectively.

Methods

The fluorine components of interest were sodium monofluorophosphate and sodium fluoride. The following methods were used to determine their concentrations in the aqueous phase of the dentifrices.

Total fluorine determination

The total fluorine in the aqueous phase was determined by a slightly modified GLC method described by Cropper and Puttnam (4).

Ten grams of dentifrice were homogenized with 20.0 ml of distilled water, and weighed. The slurry was then centrifuged to separate the solid phase which contained the precipitated calcium fluoride. Then, 1.0 ml of supernatant water phase was transferred to a screwcapped test tube and 0.1 g of fluorine-free calcium carbonate and 0.2 ml of concentrated hydrochloric acid were added and the cap was rapidly screwed on. The mixture was shaken and warmed in a boiling water-bath until all the calcium carbonate had reacted. After cooling under tap water, 0.2 ml of trimethylchlorosilane was added, and the mixture thoroughly shaken. The fluoride ions, both free and liberated from the hydrolysis of the monofluorophosphate, were then converted to trimethylfluorosilane:

$$(CH_3)_3SiCl + F^- \rightarrow (CH_3)_3SiF + Cl^-.$$

One millilitre of benzene, containing normal pentane as internal standard (2.00 ml $n\text{-}C_5H_{12}/l$), was added, and the mixture was shaken for about 2 min and allowed to stand for half an hour.

The components of the supernatant benzene phase were then separated by GLC under the following conditions: Column: 180 cm × 3.2 mm, 6% OV-1 on acid-washed, DMCS-treated Chromosorb W, 60/80 mesh. The

* Barnängen AB, Stockholm, Sweden.

column temperature was 60°C and the carrier gas flow rate 15 ml min^{-1}.

A typical chromatogram is shown in *Fig. 1*. The molar ratio trimethylfluorosilane and normal pentane was calculated and plotted against the peak height ratio of the same components (see *Fig. 2*). A good linearity was obtained (regression coefficient 0.9998).

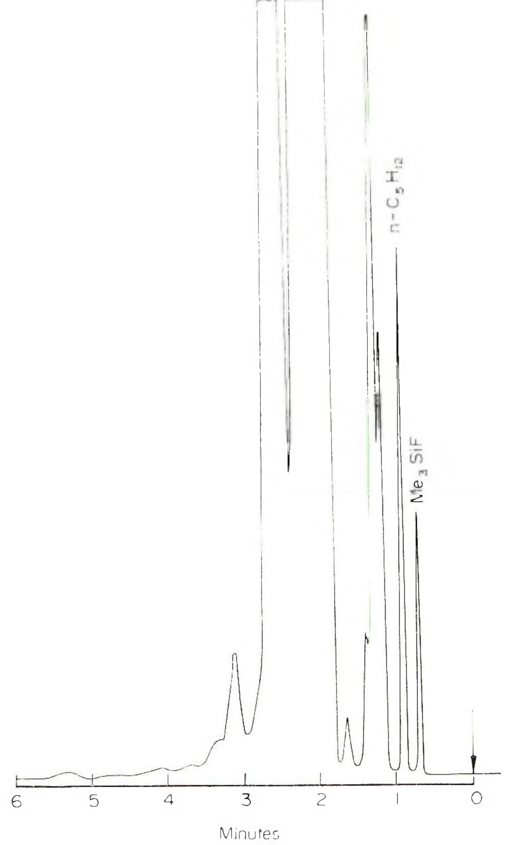

Figure 1. GLC chromatogram of the benzene phase.

Free fluoride determination

The fluoride concentration in the aqueous phase was measured directly with a fluoride-selective electrode (Orion, model 94–09). About 1.0 g of dentifrice was diluted to 50 ml in a total ionic strength adjustment buffer (TISAB) (5). A linear correlation is obtained between the electrode potential and the logarithmic concentration of fluoride ions (see *Fig. 3*).

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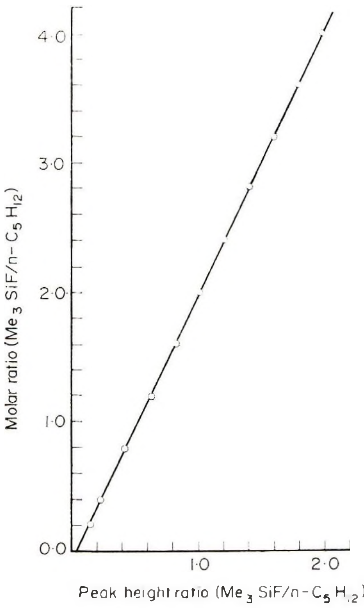

Figure 2. The correlation between the molar ratio of trimethylfluorosilane and normal pentane and the peak height ratio of the same components.

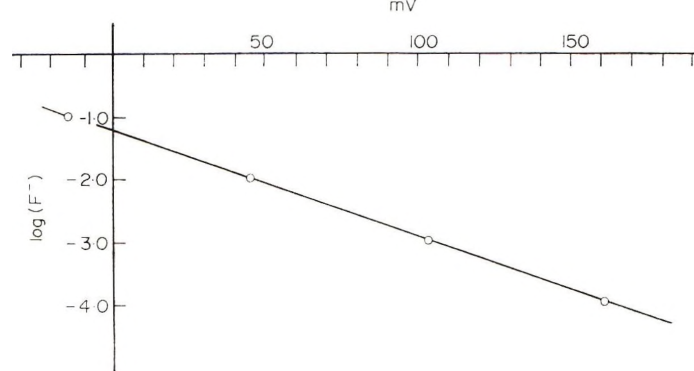

Figure 3. Fluoride electrode potential as a function of fluoride ion concentration in a TISAB-water solution.

Monofluorophosphate determination

The monofluorophosphate concentration was then taken as the difference between total fluorine and fluoride concentrations of the liquid phase. Note that the inactive calcium fluoride is not included in the total fluorine concentration.

The results have been expressed in μmol g^{-1} dentifrice.

Test dentifrices

The dentifrices used had the following basic formulation:

Calcium carbonate, precipitated	36.1%
Water	39.6
Glycerol (86%)	18.0
Sodium carboxymethyl cellulose	1.3
Lauryl alcohol sulphate	1.4
*Aerosil 200**	1.2
Saccharin, sodium salt	0.1
Flavour	1.4
Sodium monofluorophosphate	See below
Sodium fluoride	See below

The fluoride components were added as either sodium monofluorophosphate, sodium fluoride or a mixture of the two, to give the added concentration to the dentifrices (*Table I*).

Table I

Dentifrice	F (μmol g^{-1})	PO_3F^{2-} (μmol g^{-1})
A	53.8	—
B	53.8	—
C	41.3	12.9
D	28.9	25.9
E	16.3	38.6
F	4.0†	51.6†

† Only commercial sodium monofluorophosphate added.

The total amount of fluorine was about 1000 ppm (52.6 μmol g^{-1}). The fluoride concentrations include impurities of sodium fluoride from the added monofluorophosphate.

A 10% slurry in distilled water of all the dentifrices had a pH = 9.5.

* Degussa, Frankfurt/M, Germany.

Results

The observed changes in the fluorine components are due to two reactions which occur in the dentifrice during the storage.

Lang (6) has found that in alkaline solutions the monofluorophosphate ions dissociate according to reaction (I):

$$PO_3F^{2-} + OH^- \rightarrow F^- + HPO_4^{2-} \quad (I)$$

By this reaction fluoride ions are liberated in the water phase.

Fajans and Steiner (7) on the other hand found that ions which form an insoluble or slightly soluble salt with the opposite charged ions in the crystal lattice of a solid body will be strongly adsorbed on its surface. This means in this case that the fluoride ions added or liberated from the monofluorophosphate will react with the surface of the calcium carbonate crystals forming insoluble calcium fluoride. This is an equilibrium reaction.

$$2F^- + CaCO_3(s) \rightleftharpoons CaF_2(s) + CO_3^{2-} \quad (II)$$

The value of the conditional equilibrium constant

$$K^1 = C^1_{CO_3}/(C^1_F)^2$$

is dependent on the environment.

In a dentifrice with the fluorine added as sodium fluoride the free fluoride content in the water phase will decrease until the equilibrium of reaction (II) is reached. The fluoride content in the water phase will then remain constant. This effect is clearly seen in *Fig. 4*. However, addition of sodium monophosphate will change the total fluoride behaviour.

The dissociation of monofluorophosphate ions is evidently a rather slow reaction. When according to reaction (II) more fluoride ions are liberated by the sodium monofluorophosphate according to reaction (I) the fluoride ion content of the water phase will then either decrease or increase depending on the relative rates of the two reactions, which in fact is strongly dependent on the concentration of monofluorophosphate and free fluoride ions respectively. A high ratio of monofluorophosphate to fluoride ions, will cause an increasing free fluoride ion content in the water phase (see toothpaste F, *Fig. 8*). At a low ratio the fluoride ion content decreases, passes through a minimum, probably at the time when the equilibrium of reaction

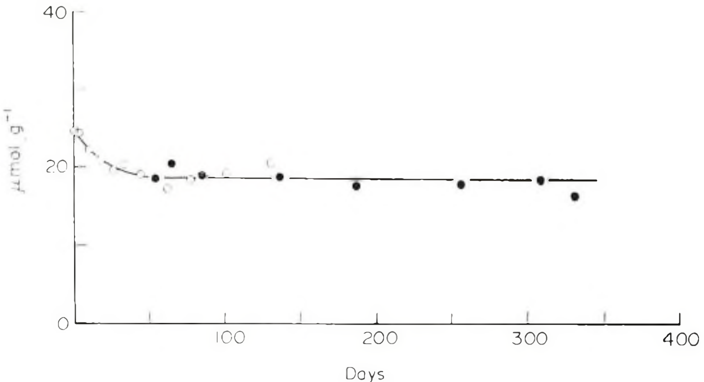

Figure 4. Changes of free fluoride ions in test dentifrices A (●) and B (○) during storage.

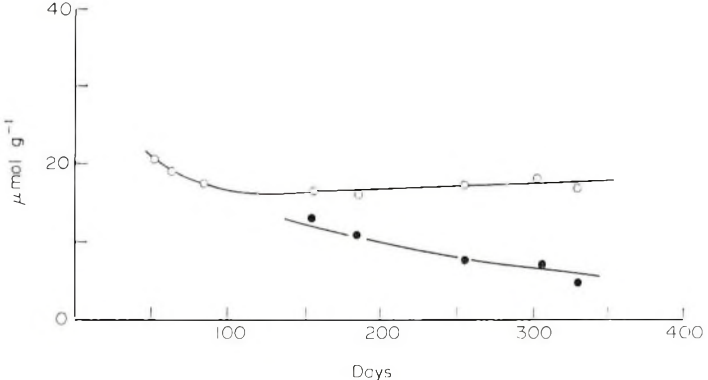

Figure 5. Changes of free fluoride (○) and monofluorophosphate (●) ions in test dentifrice C during storage.

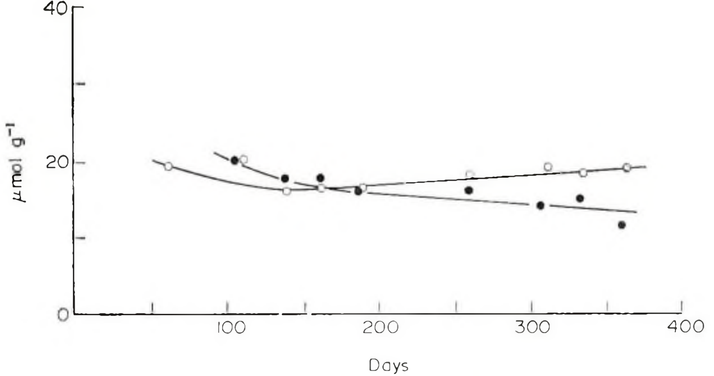

Figure 6. Changes of free fluoride (○) and monofluorophosphate (●) ions in test dentifrice D during storage.

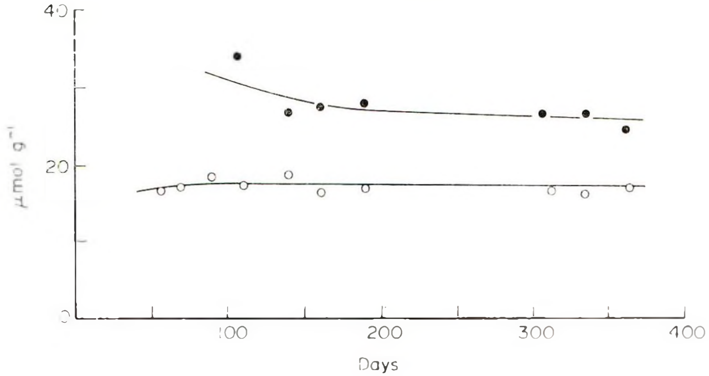

Figure 7. Changes of free fluoride (○) and monofluorophosphate (●) ions in test dentifrice E during storage.

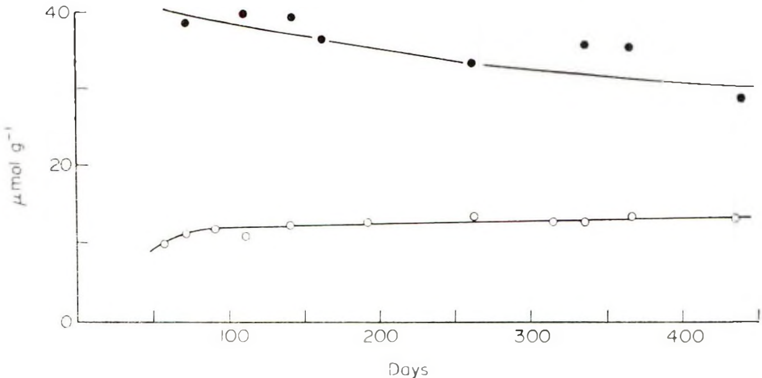

Figure 8. Changes of free fluoride (○) and monofluorophosphate (●) ions in test dentifrice F during storage.

(II) is reached and then starts to increase (see C and D, *Figs. 5 and 6*). In toothpaste E, with an initial ratio monofluorophosphate : fluoride of 2 : 4, the fluoride content was constant during the first year (*Fig. 7*).

According to reaction (I) the monofluorophosphate content decreases in all test toothpastes where it is present (*Figs. 5–8*).

The results clearly demonstrate that it is possible to add sodium fluoride to a dentifrice containing calcium carbonate without getting a total inactivation of the free fluoride ions. As the rate and equilibrium constants of reactions (I) and (II) are dependent on the environmental conditions no assumptions can be done about the fluoride ions behaviour in dentifrices with different basic formulas.

ACKNOWLEDGMENTS

This work is part of a research programme on dentifrices performed on behalf of one of the member companies of the Kabi Group, Grummebolagen.

We wish to thank Dr John Holmberg, head of the Surface Chemistry Laboratory at the Research Department of the Kabi Group, for his encouraging advice and valuable discussions. We also wish to thank Professor Bertil Åberg, the Director of Research of the Kabi Group, for his interest in our work and the permission to publish the results, and Mr William Hilton-Brown, who has revised the English text.

(*Received: 30th March 1973*)

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The chemistry of human hair cuticle—I: A new method for the physical isolation of cuticle

J. A. SWIFT and B. BEWS*

Synopsis—The CUTICLE of human HAIR has been isolated in bulk by a new method involving vigorous agitation of fibres in water. The cuticle fractions have been shown to be of high morphological purity using various techniques of ELECTRON MICROSCOPY. The significance of AMINO ACID ANALYSES is discussed.

Introduction

Despite the fact that any toiletry treatment of human hair must involve direct contact with the outer cuticle layer, either cleaning its surface, depositing materials on it or merely diffusing through it, very little is known about the chemical composition of this layer. The present series of papers describes studies carried out to elucidate the chemical nature of this component and thereby to improve our understanding of its interaction with cosmetic agents.

Cuticle structure

At the root end of human scalp hair the cuticle is about 3 μm thick and sheaths the underlying solid cylindrical core of cortex. It consists of a series of cellular sheets each about 0.3 μm thick and in the form of a segment from a truncated conical shell about 40–60 μm in length and breadth which

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overlay each other in a way analogous to that in a stack of disposable plastic cups. Under the transmission electron microscope some five to ten cuticle cell layers can be seen in a transverse section of the fibre (*Fig. 1*). From such electron micrographs it is clear that each cell is laminated, being divided into two principal components, the exocuticle (at the greater radius from the fibre axis) and the endocuticle (at the lesser radius). Similar sections but stained with silver salts (*Fig. 2*) show the presence of two further laminae within each cell: the A-layer which is a sub-component of the exocuticle and is at the periphery of the cell on the outer-facing aspect and the inner-layer which is between the endocuticle and the periphery of the cell on the inner-facing aspect.

From electron histochemical observations (1) it has been established that the exocuticle, A- and inner-layers are exceedingly rich in cystine whereas there is little or no cystine in the endocuticle. In addition, since the endocuticle stains intensely with dodecatungstophosphoric acid (a trivalent anion of a heteropoly acid) and with uranyl acetate, we believe this indicates that acidic and basic amino acid residues are in relatively high concentration in this component.

Each cuticle cell is separated from adjacent cells by a cell membrane complex and the regularity of internal structure of the complex can be seen at high magnification in transmission electron micrographs of dodecatungstophosphoric acid-stained transverse sections of hair (*Fig. 3*) (2). The overall thickness of the complex lamina is about 28 nm. The central dark-staining portion, which is about 18 nm thick, is considered to be an intercellular cement composed of protein or polysaccharide, and is generally referred to as the delta-band. On either side of this is a narrow layer some 2.5 nm thick which cannot be stained and is thought to be the lipid component of the cell membrane from each cell (2).

Electron microscope observations of human hair cuticle clearly reveal a multiplicity of sub-components. Considerable variation in the chemical composition of these various laminae may therefore be expected.

Isolation of cells

Many methods have been described for the isolation of different cell types from mammalian keratin fibres. A large number of these have relied upon chemical treatments of the fibre (3–6) and are clearly to be avoided where further detailed chemical studies are required of the isolated cells. In the case of fibre cuticle Wolfram and Lindermann (7) have recently

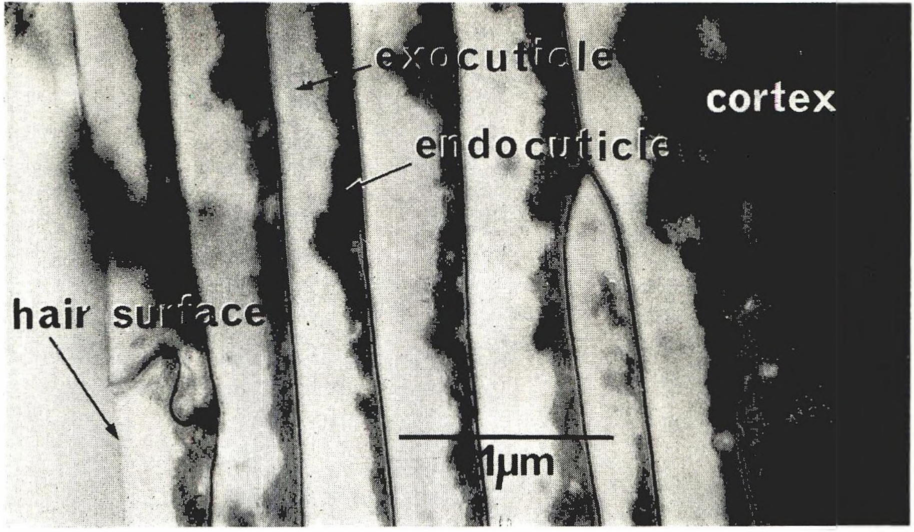

Figure 1. Transverse section of human hair cuticle. Phosphotungstic acid stain.

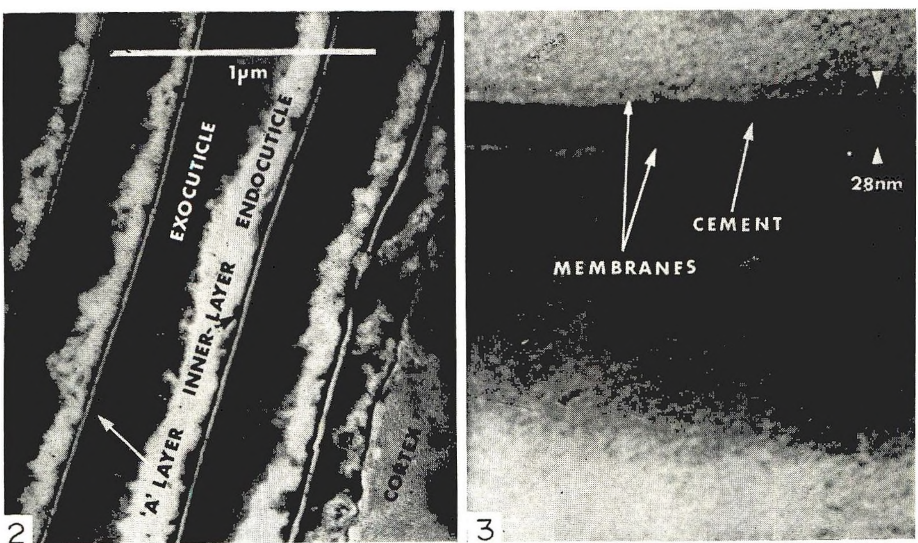

Figure 2. Transverse section of cuticle. Silver-methenemine stain.

Figure 3. High magnification electron micrograph of sectioned cuticle cell membranes. Phosphotungstic acid stain.

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described a method for the physical isolation of this component from human hair. That this method, which involves the razor blade scraping of hair tresses, yields good cuticle preparations is manifested by the considerable differences in amino acid composition between the cuticle and the whole hair. This method is rather laborious and tends to yield only relatively small quantities of assuredly pure cuticle from large amounts of hair. Much larger quantities of physically isolated cuticle are desirable where further fractionation is envisaged.

In the course of investigations on the surface architecture of human hair, vigorous shaking of hair in water was found to release small cuticle fragments into suspension (8). The present paper is concerned with the improvement of this shaking technique to yield large amounts of pure cuticle suitable for fractionation studies.

Materials and methods

The following shaking equipment was used in the course of our experiments to investigate the release of human hair cuticle into aqueous suspension: (1) a 100 W MSE ultrasonic probe with a 9 mm tip velocity transformer, (2) a Mickle tissue disintegrator (Mickle Laboratory Engineering Company), (3) a Silverson multipurpose mixer/homogenizer with a micro 15·9 mm head, (4) a Cenco-Virtis 45 macrohomogenizer, (5) a Gallenkamp 'wrist-action' flask shaker type SD 110 and (6) a Baird and Tatlock 'elliptoid' flask shaker type 330/0012.

Hair cut from the first 10 cm at the root end of ether-degreased switches of untreated Italian hair was used in the experiments. The conditions for shaking hair in water using the above equipment were varied and included variation in agitation speed, flask size and shape, quantity of water in each flask, water : hair mass ratio and average length of fibres. The turbidity of the water at various time intervals in relation to the amount of hair shaken was taken as a rough guide to the efficiency of release of hair fragments into suspension and in some cases the actual percentage yield of fragments was determined gravimetrically after filtration.

Bulk hair was separated from suspended small fragments by filtering through a 100 mesh stainless steel grid and cleared of small fragments by successive gentle agitation and filtration on the steel grid. Samples of this hair were dried, attached to a scanning electron microscope stub with double-sided Sellotape and vacuum coated with a thin layer of carbon and

silver prior to examination in a Cambridge Stereoscan Mk. II scanning electron microscope (8).

Hair fragment suspensions were centrifuged at 38 000 *g* for 60 min and the supernatant liquid removed. These residues were either dried *in vacuo* over phosphorus pentoxide in preparation for amino acid analysis and transmission electron microscopy or resuspended in a small volume of water and prepared for examination in the scanning electron microscope. For the latter purpose a drop of suspension was allowed to dry on the emulsion surface of a small piece of fixed, washed and dried photographic plate attached to a scanning electron microscope stub. This procedure ensured that all the fragments were lightly attached to the mount. Such specimens were vacuum-coated and examined in the scanning electron microscope in the normal manner.

Some of the dried fragment residues were intimately mixed with Spurr's resin (Taab, Reading) (9) and introduced into Beam capsules (LKB Produkter). These were allowed to remain at room temperature for 24 h and were then polymerized overnight at 65°. Thin sections (60–100 nm) of the embedded fragments were cut on a Porter-Blum MT-2 ultramicrotome with a glass knife and mounted on 100 mesh gold electron microscope grids (Polaron) covered with a thin collodion/carbon supporting membrane. Some of the section-laden grids were stained for 20 min at room temperature in a solution of 0.1 M silver nitrate to which 0.880 ammonia had been added dropwise until the precipitate had just dissolved and then rinsed for 30 s in distilled water. Other grids were stained with a filtered 2% w/v solution of dodecatungstophosphoric acid (PTA) in 50% ethanol for 3 h at 65° and then rinsed in distilled water. In some cases the silver-stained sections were also stained with PTA. The various stained grids were examined in a JEM 7 transmission electron microscope at 80 kV and using a 50 μm objective aperture.

Amino acid analyses of dried hair fragment preparation and samples of the original hair were carried out with a Technicon Autoanalyser by the Wool Industries Research Association (Leeds).

Results and discussion

The ultrasonic method proved ineffective for producing hair fragment suspensions because the generation of air bubbles caused the hair to float at the top of the irradiation cell. A very slow rate of disintegration of wool during sonication in water has also been reported by Bradbury (10). Both

the high speed rotary homogenizers (Silverson and Cenco) yielded large amounts of suspension within a few minutes at high speed (2% by wt of hair in 5 min) but unfortunately it was clear from the scanning electron microscope examination of the fibres and fragments that considerable breakdown of the cortex had occurred. Conditions could not be found where this breakdown did not occur.

Shaking with the Mickle disintegrator did release clear cuticle fragments into suspension but only about 10 mg of isolated cuticle could be prepared per day because of the limited working volume. The wrist action shaker (Gallenkamp) also yielded pure cuticle suspensions and under the best conditions (1.25 g of hair cut to 2 cm length plus 50 ml of water in each of four 100 ml round-bottomed stoppered flasks shaken at about 15 Hz with an amplitude of 7 mm along the axis of the flasks) about 20 mg of cuticle could be prepared per day.

Greatest success was obtained with the elliptoid shaker (Baird and Tatlock). Using 0.3 g of hair cut to 2 cm length plus 15 ml of water in each of four 30 ml *Sterilin* plastic disposable 'Universal' bottles shaking at 30 Hz, some 50 mg of cuticle could be prepared in as little as 2 h. The yield of suspended material from successive shaking periods is shown in *Fig. 4*.

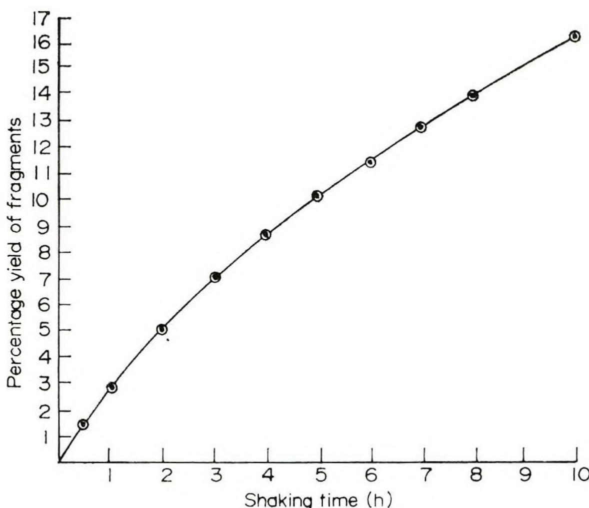

Figure 4. Graph showing percentage yield of hair fragments with time.

From area measurements carried out using electron micrographs of hair sections, about 12% of human hair root end fibres may be classified as

cuticle. From *Fig. 4* there is clearly no abrupt change in the rate of fragmentation which might correspond to the complete removal of cuticle. On the other hand there is a gradual reduction in the rate of release of fragments perhaps indicating that cortex breaks down slightly slower than the cuticle. Bearing this in mind we have chosen to accept those fragment fractions obtained after $2\frac{1}{2}$ h shaking with the elliptoid shaker as containing only cuticle—our electron microscope observations confirmed that this fraction is not contaminated with cortex.

The mechanism whereby cuticle is released into suspension is of some interest. To understand what happens to hair when it is soaked in water, the surface architecture of air-dried fibres and fibres, which after immersion in water were rapidly frozen and freeze dried, have been compared in the scanning electron microscope. Whereas the cuticle cell surfaces of air-dried hairs are normally fairly smooth (8), the freeze-dried fibre cuticle appears to be quite bloated (*Fig. 5*) (this can usually be seen very clearly in stereo-pair electron micrographs). Since the exocuticle-, A- and inner-layers are highly cross-linked by cystine (1) these components cannot be expected to swell appreciably in water. On the other hand swelling of the endocuticle can be expected since it contains virtually no cystine but may contain higher than average concentrations of acidic and basic amino acid residues. This differential swelling is summarized in the schematic diagram of *Fig. 6*.

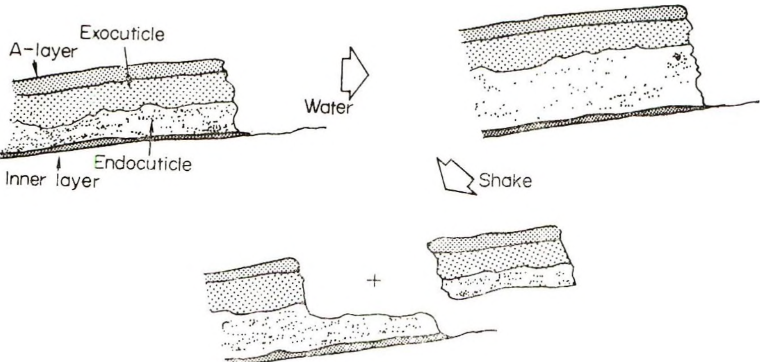

Figure 6. Schematic diagram illustrating the swelling and fragmentation of cuticle.

In the elliptoid shaker the hair forms a loosely tangled mat which moves about only slowly, but the water, or rather the air-in-water mixture, is thrust rapidly up and down through the mat. Under these conditions the highly turbulent flow of water over the hair, coupled with the high surface

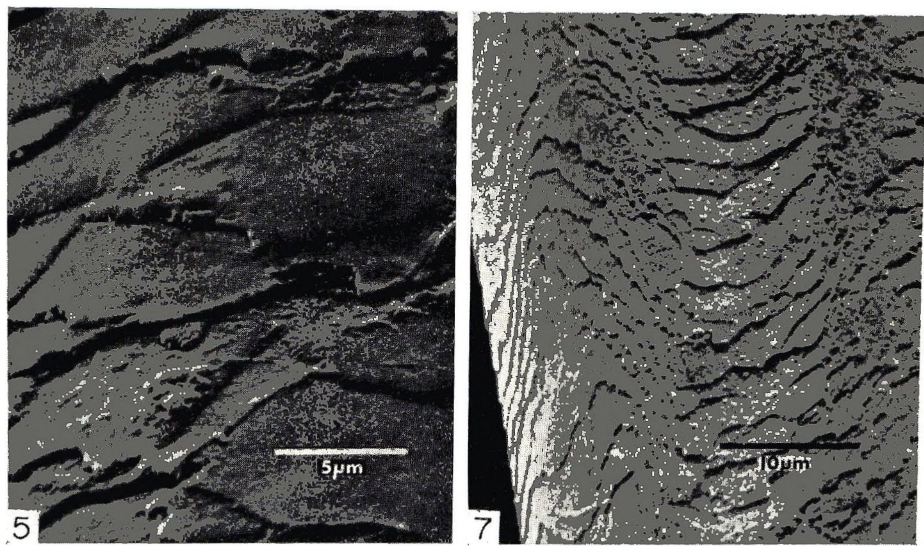

Figure 5. Scanning electron micrograph of wetted and freeze dried hair showing swollen cuticle.

Figure 7. Hair after 2 h shaking.

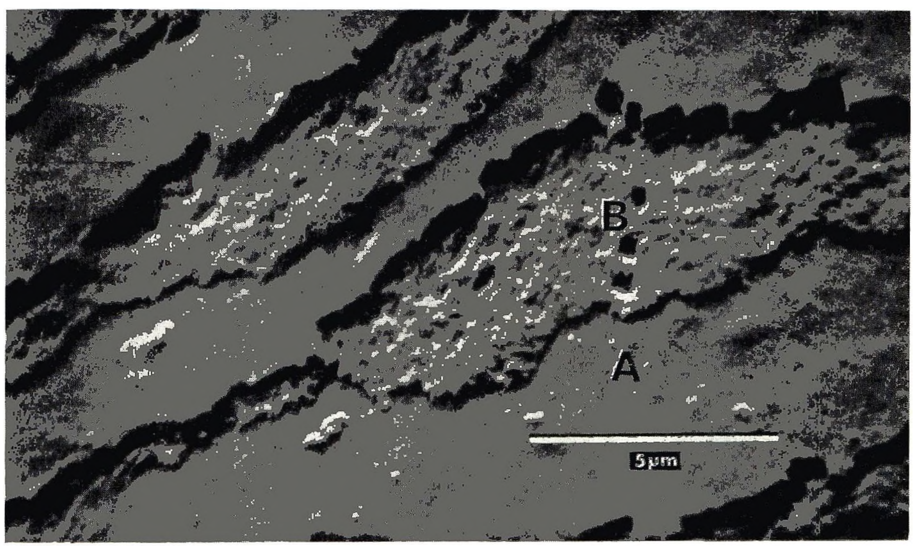

Figure 8. Scanning electron micrograph showing the granular deposit remaining on the hair after shaking.

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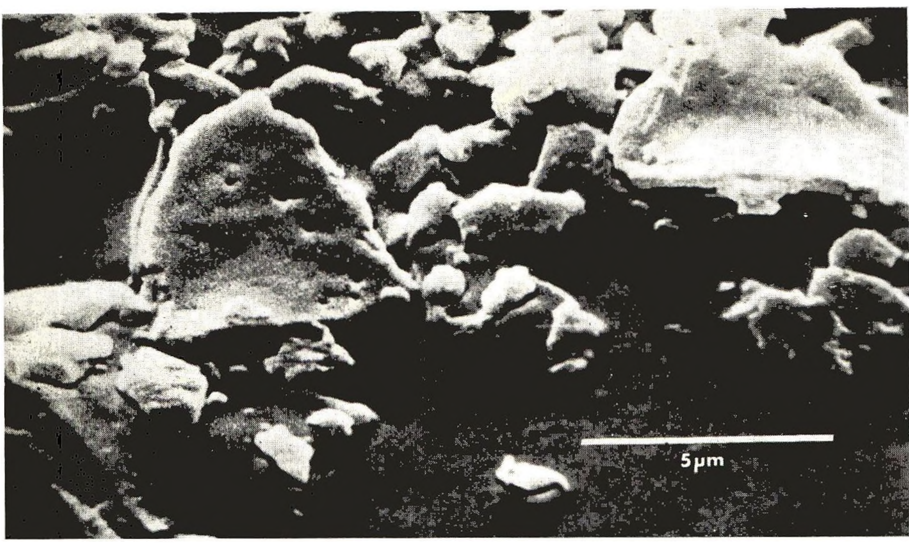

Figure 9. Scanning electron micrograph of the fragments produced after $2\frac{1}{2}$ h shaking.

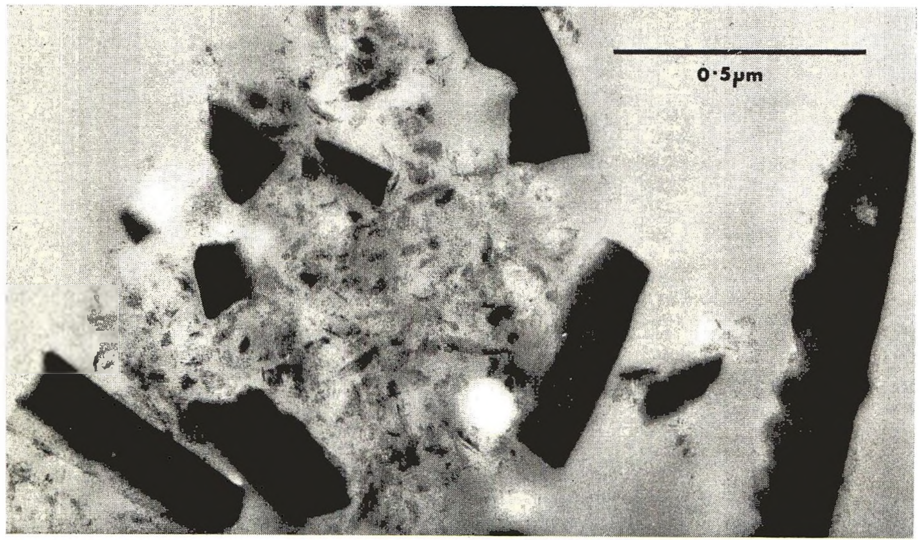

Figure 10. Transmission electron micrograph of a section of the fragments. Ammoniacal silver/phosphotungstic acid stain.

forces associated with the air in water mixture, appears to be sufficient to strip off the cuticle at the swollen endocuticular layer (*Fig. 6*). Since the exocuticle is so highly cross-linked and brittle, pieces of cuticle will snap off. Such a mechanism is consistent with the electron microscope observations of cuticle fragment morphology.

The surface architecture of hairs after shaking on the elliptoid shaker was examined in detail with the scanning electron microscope. Examples of hairs shaken for 2½ h are shown in *Figs 7 and 8*. Whereas the cuticle is eroded away evenly over the whole circumference of circular or slightly elliptical section fibres (the majority of fibres) some interesting wear patterns were seen on the small proportion of irregular fibres (*Fig. 7*). Here, the cuticle is more rapidly removed from ridges than from troughs on the fibre surface. Similar scale patterns have been observed in other irregular fibres such as pubic or nostril hair and are undoubtedly due in these cases to natural wearing of the fibre surface. At high magnification the scale surfaces of the shaken hair are relatively smooth for a short distance close to their distal ends (marked A in *Fig. 8*) but beyond this up to the edge of the next overlying cell the scale surface is granular (B in *Fig. 8*). This granular layer is undoubtedly endocuticle which remains after the exocuticular portion of the cell and some adhering endocuticle has been stripped away in the shaking process. It seems likely that the granular layer is also eroded away as much smaller fragments, leaving the clearly exposed exocuticular or membrane surface of the underlying cell.

Under the scanning electron microscope the fine particulate material produced by shaking is seen to be composed mainly of small platelets about 0.2 μm thick and up to 5 μm in diameter and a relatively structureless finer deposit (*Fig. 9*). From sections of this material examined under the transmission electron microscope it was evident that the platelets are composed of exocuticle and A-layer with small amounts of adhering endocuticle (*Fig. 10*). Considerable amounts of finely particulate material about 80 nm in diameter were also encountered. Most of this latter material stained intensely with dodecatungstophosphoric acid and was therefore almost certainly of endocuticular origin. A small proportion of these fine particles was stained by ammoniacal silver and is therefore probably derived from the inner layer of the cuticle. Sometimes fine filaments up to 2 μm in length and about 15–20 nm in thickness were seen in the phosphotungstate stained sections. These were probably delta-band and attached unstained membrane derived from the intercellular membrane complex of the cuticle. There was no evidence of fragments which could have been derived from

the hair cortex. From the foregoing the material released into suspension after 2½ h under the defined conditions of shaking on the elliptoid shaker is clearly to be regarded as virtually pure cuticle.

Amino acid analyses of our 2½ h cuticle fraction and that of samples of the original hair are shown in *Table I*. Included in this table for comparison are Wolfram and Lindemann's results (7) which have been recalculated in terms of moles of each component/1000 mol of total amino acid. For all the amino acids except proline there is exceedingly good agreement between the two sets of cuticle analyses. There is some variation between the two separate analyses for whole hair but this is probably accounted for by natural variations from one individual to another (11). The cuticle evidently contains significantly higher concentrations of serine, proline (in the case of Wolfram and Lindemann's results), glycine, alanine, valine, cystine and lysine and lower concentrations of aspartic acid, threonine, glutamic acid, leucine and arginine than the cortex. In terms of the cystine content it is interesting to note that from electron histochemical observations we have

Table I.

Amino acid composition of human hair and human hair cuticle

Amino acid	Content in mol/1000 mol total amino acids analysed			
	Present results		Wolfram and Lindemann's results (7)	
	Cuticle	Hair	Cuticle	Hair
Cysteic acid	8.8	3.4	6.2	4.1
Aspartic acid	30.9	57.7	31.4	51.6
Threonine	44.1	74.5	43.1	71.7
Serine	168.9	115.2	170.3	125.1
Glutamic acid	92.5	129.5	88.7	118.5
Proline	64.6	68.0	94.2	76.1
Glycine	97.4	61.1	87.5	56.5
Alanine	56.6	46.2	52.3	44.9
Valine	68.7	49.8	67.4	52.4
½ Cystine	202.2	167.3	196.7	185.6
Methionine	4.8	2.1	4.1	1.7
Isoleucine	20.3	25.4	19.5	22.5
Leucine	46.2	64.9	42.3	59.1
Tyrosine	17.0	21.4	14.0	20.4
Phenylalanine	12.1	16.5	12.0	16.0
Lysine	33.1	24.9	34.6	25.4
Histidine	4.5	7.1	5.5	8.0
Arginine	27.4	65.1	30.2	60.3

found (1) that virtually all the cystine in the cuticle is contained in the exocuticle-, A- and inner-layers. Measurements of electron micrographs of sectioned cuticle showed that these components occupy about 65% of the total area of the cuticle. The average concentration of $\frac{1}{2}$ cystine in these components may therefore be expected to be of the order of $200 \times \frac{100}{65} =$ 310 mol/1000 mol of amino acid. Such proteins containing nearly 1 in 3 amino acid residues as $\frac{1}{2}$ cystine are likely to be extremely tough.

From the present observations the compositions of the various sub-layers of the cuticle will prove of further interest. The present method for isolation of the cuticle should be of considerable value as a preliminary to the fractionation and analysis of these sub-layers.

Acknowledgments

We are indebted to Dr J. C. Fletcher of the Wool Industries Research Association for the amino acid analyses of our materials and to our colleague Dr A. C. Brown for his work on freeze-dried fibres.

(Received: 15th March 1973)

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The substantivity of cosmetic ingredients to the skin, hair and teeth

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Presented on the 12th February 1973 in London, at the Symposium on 'Some surface chemical aspects of cosmetic and toiletry products', organized by the Society of Chemical Industry and the Society of Cosmetic Chemists of Great Britain.

Synopsis—SUBSTANTIVITY conveys the idea of prolonged association between a material and a substrate, an association which is greater or more prolonged than would be expected with simple mechanical deposition. This review is intended to discuss the advantages and disadvantages of substantive effects, various ways of achieving substantivity and methods for its detection and assessment.

THE NEED FOR SUBSTANTIVITY

Lipstick is a typical example of decorative make-up which is left at the site of application for many hours. The effect of pigmentation is mainly achieved by purely mechanical deposition.

In much the same way, various 'toiletries' are applied in a matter of seconds, or perhaps for a minute or two; we then expect them to have an effect throughout the whole day or even longer. However, such preparations —for example, shampoos and toothpastes—are often rinsed away with copious amounts of water immediately after application. Sunscreens are not deliberately rinsed off in this way, but they are expected to retain their screening effect even after the body has been completely immersed in seawater.

So it is evident that substantivity could be extremely helpful in the cosmetic and toiletries field, to assist in providing long-lasting benefits.

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Clearly, the duration of effect may be even more important with so-called 'active' constituents.

In practice, the need for clear evidence to show a substantive effect often becomes most obvious when the claims for a new cosmetic have to be justified to an adjudicating body. For example, an advertisement for a deodorant may suggest that one application will guard against perspiration odour for several hours; evidence to substantiate such a claim will almost certainly be requested.

Another example of the need for substantivity is the inclusion of an anticaries agent in a dentifrice. de Boever and Muhlemann (1) have shown that carious damage or dental decay is probably the result of *long-term* maintenance of a low pH in the layer of plaque on the enamel. It follows from this that a prophylactic agent to counteract the acidity of the plaque, has to be persistent during a major part of the interval between toothbrushings.

An antibacterial system, to control the plaque bacteria, also needs to show persistence in the oral cavity. This might be achieved by way of substantivity to the dental enamel or to its organic coatings or to the oral soft tissues. Löe, Mandell, Derry and Schiott (2) have claimed successful results with chlorhexidine mouthrinses in plaque control and in calculus or tartar prevention. Recently, a clinical study on young adults has also shown significant reduction in dental plaque, using a mouthrinse containing 0.035% of alexidine, an ethyl hexyl-bisguanide (3). One of the reasons for selecting this compound was its adsorption to dental enamel, which presumably helped to ensure its prolonged antimicrobial action.

On the other hand, the usual idea that antimicrobial activity is the main requirement for dealing with the dental plaque has recently been challenged. A group working at the University of Tennessee (4) prepared a series of compounds representing dissected segments of the chlorhexidine molecule. They then proceeded to show that the adsorption of the chlorhexidine congeners on to hydroxy-apatite is closely matched by their surface activity in water or in a hexane-water system. In the view of these authors, antimicrobial activity may be less important than the surface-active effect of chlorhexidine; they suggest that this may help to dissipate cohesive and adhesive forces in the dental plaque.

The hair, once it has emerged from the scalp, is no longer a living tissue, but the concept of substantivity is still relevant. Hair-dyeing is an obvious example of a substantive process. The distinction between temporary, semi-permanent and permanent hair dyes simply indicates different degrees of substantive effect.

These illustrations may suggest that substantivity is always a desirable property but this does not necessarily follow. If a human tissue selectively removes a component from a topical application, perhaps with some degree of protein-binding or alteration of cell membranes, there is a distinct risk of toxicity. For example, the fact that cationic surfactants are highly substantive to the tissues is undoubtedly responsible, in part, for the severe eye irritation they can cause (5). So it was very surprising to see a recent article in a Sunday newspaper on shampoos. The writer recommended using cetrimide solution, without any reference whatever to the dangers, in preference to the properly-tested shampoo brands on the market.

The systemic toxicity of a topical application depends on the quantity applied, skin penetration, distribution in the body, metabolic transformation and excretion, in addition to any specific toxicity at a particular site. Whether toxic signs are actually seen in normal use of a product will depend not only on its *inherent* toxicity, but also on the balance between factors, such as the rates of skin penetration and excretion. This balance will determine whether toxic substances accumulate in the body. Enhanced retention of cosmetic ingredients on the body surfaces—that is to say, substantivity—will tend to increase the degree of exposure or, in other words, the effective dose-level. On the other hand, if substantivity is achieved in such a way that the 'active' constituent is localized on the skin *without promoting absorption*, toxicity could be reduced. Such localization will help excretion to keep pace with systemic absorption. The dynamic aspects of safety-in-use cannot usually be forecast on theoretical grounds. Hence there is likely to be a demand for increasingly more detailed toxicological evaluation. In fact, the study of absorption, blood levels, tissue distribution and excretion is now seen as being almost as important as the determination of toxicity itself. It should be recognized that, whenever substantive properties are sought by modifying the *surface activity* of a formulation, there could well be a risk of promoting skin penetration and raising blood levels. This is one reason why it is often contended that the toxicity of a formulation cannot be fully assessed from data concerning the individual ingredients.

Means of achieving substantivity

Substantivity involves mechanisms such as adsorption, ion exchange and chemical interaction. These mechanisms are too well-known to require explanation, but some aspects perhaps call for further discussion. For

example, account should be taken not only of the surface activity of a product but also that of the skin and its secretions. The skin is, in fact, often regarded as being bathed in an emulsion of sebum, sweat and epidermal remnants. The properties of this substrate will vary from the normal, for example, if a cosmetic is generally applied soon after the skin has been washed with soap and water or if it is used immediately after shaving.

As already indicated, a clear example of substantivity is the relative ease with which cationic materials can be deposited on body tissues and retained there. There are many anionic sites available on the substrate with numerous carboxy groups available for interaction. A recent publication, by Janet Woodard (6) of Dow Chemical, describes the substantive properties of the polyethylenimines. These behave as cationics in an aqueous environment, owing to the many amino-nitrogens which become positively charged, especially at a pH of about 4. The addition of a polyethylenimine to a shampoo is claimed to result in uptake on the hair—especially on damaged hair—and to improve combability and manageability.

It is of interest to consider also the behaviour of antiperspirants. In order to work at all, they must presumably have a substantive effect somewhere in the eccrine sweat gland or duct. Kligman (7) has shown that dimethyl-sulphoxide (DMSO), a well-known agent for enhancing skin penetration, increases the antiperspirant effect of the customary aluminium salts. This finding is of theoretical interest although DMSO is probably too toxic for inclusion in an antiperspirant formulation in practice. In another paper (8), Kligman maintains that the aluminium salts inhibit sweating by converting the eccrine sweat duct into a so-called 'leaking hose'; this prevents the sweat from reaching the skin surface. Others have tried to explain antiperspirant activity in terms of inhibiting the secretory portion of the eccrine gland, or blocking the opening of the eccrine duct. So, the fundamental nature of antiperspirant activity is still open to question. The lack of theoretical clarity makes it difficult to understand what kind of substantive action is really needed to improve antiperspirant performance, but obviously a long-term effect is desirable.

As mentioned already, anticaries action may well depend on the principle of substantivity. Fluoride in the diet or in the drinking water is principally of benefit to infants and children up to about 8 years of age. By this time the enamel of the second dentition is almost fully formed, even though some of the permanent teeth have not yet erupted. Fluoride provided systemically during the course of enamel formation is built into the enamel as fluorapatite. Topically-applied fluoride, on the other hand, only affects the enamel surface.

Studies on fluoride uptake by Weatherell (9) and his colleagues at Leeds, suggest that soluble fluorides at the levels included in typical fluoride toothpastes only give a negligible uptake into *sound* enamel. Much greater incorporation seemingly occurs if the enamel has been partially demineralized in an acidic buffer. The effectiveness of fluoride toothpastes has been confirmed in numerous clinical trials. Weatherell's findings indicate that they may function mainly by arresting the progress of 'incipient' carious lesions; that is, these toothpastes greatly increase the fluoride content of enamel if it has been partly demineralized by acid from the dental plaque. This line of reasoning also suggests that artificial means might be employed to 'condition' the enamel surface, so that its fluoride uptake is enhanced. Katz, Muhler and Beck (10), in fact, have indicated two main ways to attempt this, (a) by formulating a dentifrice with a relatively low pH or (b) by treating the enamel with a sequestering agent for calcium ions. Some doubt is cast on the idea of conditioning the enamel by a recent paper from Aasenden, De Paola and Brudevold (11). In a clinical study on 8–11-year-olds, they observed equal protection against caries by daily rinsing with either a *neutral* sodium fluoride solution or an *acidulated* phosphate-fluoride preparation with a similar fluorine content. Enamel biopsies showed a greater uptake of fluoride from the *acidified* rinse but evidently the difference was not enough to improve the anticaries performance. So it seems rather unlikely that the anticaries effect of a fluoride toothpaste could be greatly improved by formulating an acidic or calcium-chelating preparation. In any event, the deliberate promotion of enamel demineralization in order to enhance fluoride uptake must obviously be employed with discretion, to avoid causing serious damage.

Another long-standing attempt to achieve substantivity—namely, with the silicone oils—is aimed at blocking the effects of other substances on the skin or elsewhere. For example, silicones are used in hand creams to counteract the degreasing effect of detergents. Cosmetic formulators greeted the silicones with high hopes when they first appeared. The results have often been disappointing but the fault may have been due to unsuitable formulation, rather than to failings in the silicones as such.

The silicones have featured in a number of attempts to deal with the dental calculus problem. The aim here is not so much water-repellancy as the prevention of *crystallization* on the tooth surface. The two concepts may well be interconnected. However, the tooth surface is always exposed to an aqueous medium, the saliva, which has excellent wetting properties. A bacterial plaque is rapidly formed on the enamel. Extracellular dextrans

resulting from bacterial metabolism ensure good adhesion of the plaque. If the saliva is supersaturated with respect to calcium and phosphate, the plaque begins to calcify; at this stage it is almost impossible to avoid the attachment of crystal nuclei to the enamel. Tamas (12) ingeniously sought to augment the water-repellent effect of the silicones by including an organo-titanium compound, as used in the waterproofing of paper, e.g. *o*-butyl titanate. He believed this would improve the bond between the enamel and the silicone. It is arguable whether his composition really was capable of inhibiting calculus deposition under practical conditions; even so, the concept of using a coupling agent of the organo-titanium type is an attractive one in theory. There would seem to be considerable scope for the synthesis of molecules capable both of adsorbing or otherwise persisting on a tissue substrate and also of 'holding' the active constituent, that is, to serve as a 'bridge'. An illustration of this principle is described in a recent article by Sardo (13). The co-polymerization of an organic titanate, such as tetra-isopropyl titanate, with a dimethyl siloxane at room temperature is said to provide a hair spray resin which offers good curl retention, combing and non-flaking properties. It is also substantive enough to the hair to withstand several shampoos.

Many dyestuffs and antiseptics obviously have substantive properties. Their ability to stain tissues and to damage micro-organisms points to their substantivity, often with beneficial results; clearly there may also be a toxic hazard, by damaging human cells. The search for skin-substantive germicides led to many promising compounds such as hexachlorophene, bithionol, trichlorocarbanilide and the halogenated salicylanilides. In almost every instance, there have been discouraging suggestions of toxic potential, even though *harm to man* during cosmetic usage may have been rare. Such toxicity cannot be *directly* attributed to substantivity of the antibacterial but this may well represent a contributory factor.

Detection and assessment of substantivity

Because the term 'substantivity' has no precise meaning in terms of a distinctive physico-chemical property, it is hard to generalize with regard to methods of detection and assessment. The use of a radioactive isotope as a tracer ought to be ideal for qualitative and quantitative verification. The technique of autoradiography is specially useful for demonstrating substantivity; this is particularly true when a sequence of autoradiographs provides clear evidence of persistence. Tracers, however, are not without

pitfalls. There is, for example, a possibility of the tracer becoming detached from the parent substance. Tracers other than isotopes are often suspect for this reason. For example, if an oil-soluble dye is used as a means of demonstrating the substantivity of an oil to the skin, one must always consider whether the dye is preferentially taken up by the skin. Indeed, it could well be argued that tracer studies will only be convincing if we are able to demonstrate the continued association of the tracer with the parent substance. If this view is taken, the effort might just as well be devoted to developing good assay procedures for the parent substance itself.

Radio-isotopes are perhaps not so easily separated, or so suspect in consequence. Even so, they can still be misleading, because exchange with a stable atom can have the same effect of dissociating the tracer from the labelled material. An experienced radio-chemist, on the other hand, should be on the look-out for this type of spurious answer.

The most promising area for developing *in vivo* techniques to study substantive behaviour on human tissues is, undoubtedly, the use of non-radioactive or *stable* isotopes such as carbon-13, nitrogen-15 and oxygen-18. Mass spectrometry makes possible the sensitive monitoring of these isotopes. Since radioactivity is not involved, it is permissible to carry out human studies (14) even with pregnant women and with children, which would be too hazardous using, say, the carbon-14 label. The toxicity of the material labelled with a stable isotope will be no greater and no less than that of the unlabelled material. This is potentially of unique interest for cosmetics, where radiobiological studies cannot usually be justified in terms of a positive benefit-to-risk ratio. One of the main limitations on the development of stable isotope studies is the availability of a wide enough range of suitably labelled compounds. No doubt these will be forthcoming in due course and some very promising investigations will then become feasible which would be virtually impossible by current methods.

The need for a positive demonstration of persistence should be emphasized. Data are sometimes offered showing, for example, the depletion of a compound from a solution in contact with the skin; it is then argued that, since the concentration of the solute has decreased, this must have been taken up by the substrate. This type of negative reasoning is unsatisfactory, because it gives no information as to persistence. Although it would be more relevant to demonstrate by chemical assay or tracer technique that a material does persistently associate itself with the appropriate substrate, even this does not give the whole answer. Generally, it must also be shown that the intended *property* is conferred. For example, it is not enough to

show the presence on the hair of protein derived from a protein shampoo; its ability to effect a so-called 'repair' of split-ends will need to be confirmed. The *in vitro* demonstration that ingredients of a formulation can be substantive to the skin, hair or teeth is certainly of value to the researcher; such a demonstration will indicate whether biological or clinical studies are likely to give a favourable result. To convince *others*—especially adjudicating bodies— the biological or clinical evidence will generally have much greater impact.

Conclusions

Attention is often drawn to the risk of upsetting the ecology of the skin and of the oral cavity. Recent evidence (15) confirms that certain, relatively harmless, strains of bacteria on the skin may be important as a protection against other bacteria of *pathogenic* significance. The successful achievement of bactericidal or bacteriostatic activity on the skin, for cosmetic purposes, might conceivably prove quite hazardous; it might increase the risk of contracting a serious clinical infection. Substantivity, in other words, is a feature involving benefit and risk and we must be fully aware of both possibilities.

Cosmetic science today is facing a powerful challenge. On the one hand, we are accused of charging high prices for products offering little benefit. On the other hand, the use of relatively potent ingredients generates problems on the question of toxicity. There is no easy way out of this dilemma, but it does seem essential to seek ways of genuinely improved product performance, without recourse to powerful pharmacologically-active ingredients. By way of illustration, this review is intended to suggest that a thorough consideration of substantivity may well open up some promising avenues.

(*Received: 29th December 1972*)

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Specialized techniques for the analysis of cosmetics and toiletries

D. M. GABRIEL*

Presented on the 7th December 1972 at the Royal Society of Arts to the Society of Cosmetic Chemists of Great Britain.

Synopsis—It is only during the last century that scientific principles have been applied to the development of cosmetics and toiletries, but during the same span of time there have been dramatic advances in ANALYTICAL TECHNIQUES, many of which have been applied to solve particular analytical problems associated with this industry.

Modern analysis is generally a two stage procedure—first the SEPARATION and ISOLATION of the various components of interest followed by CHARACTERIZATION, IDENTIFICATION and ESTIMATION.

Examples illustrate the use of a wide range of techniques which have been applied to the analysis of SHAMPOOS, AEROSOL HAIRSPRAYS, HAIRDRESSINGS, TOOTHPASTES, ANTIPERSPIRANT/DEODORANTS and TALCUM POWDERS.

A suggested reading list is appended.

INTRODUCTION

The origins of the cosmetics and toiletries industry lie way back in history before the 5th century B.C. when aromatics and unguents were used for the religious purposes of anointing and embalming. These odorous and oily substances were derived from natural sources but the compounders and users were unaware of their complexity. All that mattered was the skill of the compounder and the functionality of these highly valued products.

It is only during the last century that science has been applied to the development of products for the hygienic care and embellishment of the

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human body. This has given rise to a sophisticated, yet practical, toiletry and cosmetic market with world wide appeal.

As more and more scientific knowledge is acquired and applied, so the need to determine the nature and level of product ingredients becomes of paramount importance. Fortunately, the advances in analytical techniques have been as dramatic and timely as the advances in scientific knowledge.

Preliminary considerations

First we must ask 'Why do we need to analyse these products?' and this question must be answered before any analysis is considered, because the analytical route depends on the kind of information required.

Specialized techniques are expensive to acquire and run, but results not easily available by other means can be obtained and providing these results are applied judiciously, the economics can be acceptable.

The problems presented to the analyst vary from the extreme of a so-called 'complete' analysis (or perhaps a general idea of the product formula), through estimations of amounts of particular ingredients that are present or available, down to determinations of trace amounts of possibly harmful impurities.

Examples drawn from the author's experience and the literature illustrate some of the ways in which specialized techniques can be applied to the analysis of toilet preparations and cosmetics together with the kind of information that can be obtained.

The cosmetic and toiletries market consists of a range of products of different physical forms (powders, lotions, creams, pastes, dispersions, suspensions, moulded solids etc.) in a variety of packs (bottles, jars, tubes, tins, aerosols) further fragmented into products for special application to every area of the human frame whether male, female or child, not forgetting multi-purpose and unisex products.

Analytical approach

Basically there are two situations—one in which we know what we are looking for, and another in which we are dealing with complete unknowns. However, these can be considered together since in each case the substance is nearly always surrounded by a complex matrix, so direct analysis is usually not feasible because a prerequisite for most of the instrumental techniques is that the substance to be identified or estimated must be in a reasonably pure state.

This means that good separation techniques are also needed, and fortunately the development of these has kept pace with the instrumental techniques. So today's analyst follows a two-stage procedure.

(1) Separation and isolation of the various components of interest.

(2) Characterization, identification and estimation.

The separation techniques are a mixture of classical and new. Chromatography is the most versatile of the new techniques and once identification has been established has the added advantage that it can also be used for estimation. The list of instrumental techniques has been limited to those which are particularly useful for the analysis of cosmetics and toilet preparations. An even wider range has been used for background studies. Attention is given to the application of techniques and a description of the underlying principles is not included but a bibliography for further reading is appended.

It is worth noting that most of the raw materials used in this industry are not single chemical entities but are mixtures, homologous series, by-products etc. and our modern separation techniques can separate virtually everything given the right conditions. It is important to know, therefore, what components are likely to be present in raw materials before drawing conclusions about the analysis of an unknown product.

Separation techniques

New	Classical
Column chromatography	Dialysis
Ion-exchange chromatography	Diffusion
Liquid chromatography (lc)	Distillation
Gas chromatography (gc)	Solvent extraction
Gel permeation chromatography (gpc)	etc.
Thin layer chromatography (tlc)	

Characterization and identification techniques

Spectroscopy

- Ultra-violet spectrophotometry (uv)
- Visible spectrophotometry
- Infra-red spectrophotometry (ir)
- Flame emission spectrophotometry
- Atomic absorption spectrophotometry (aas)
- Fluorescence spectrophotometry

Nuclear magnetic resonance spectroscopy (nmr)
Mass spectrometry (ms)

Thermal analysis
Differential thermal analysis (dta)
Thermogravimetric analysis (tga)

X-ray
Diffraction
Micro-analysis

Polarography

Ion selective electrodes

Application of techniques

Surfactants in shampoos

Let us start at the top with hair—clean, shining, manageable hair, which automatically suggests shampoos. Most modern shampoos contain one or more mild detergents and optionally a lather booster. These surface active agents can belong to one or more of the five main groups of surfactants, namely soaps, anionics, cationics, nonionics or amphoterics.

So if the question is which surfactants are present and the amounts of each, how can the answer be obtained?

First, the species must be separated and one of the best ways is by ion-exchange chromatography. One scheme is illustrated in *Fig. 1*. An alcoholic solution of the shampoo non-volatiles is passed down three columns in series. The nonionics pass straight through and are collected in the eluent. The anionics are held on the first column, some amphoterics on the second and the cationics plus any non-volatile bases on the third.

The columns are then separated and each one eluted. The first one with 3N ammonia in ethanol and the second and third with N hydrochloric acid in ethanol.

Each fraction is then evaporated to dryness and extracted with acetone, iso-propanol etc. to remove inorganic salts. Soaps, if present, are best removed as fatty acids by acidifying and solvent extracting the shampoo non-volatiles prior to ion-exchange chromatography.

The fractions thus obtained can be further examined. First it is useful to monitor each fraction by means of its infra-red spectrum since this is

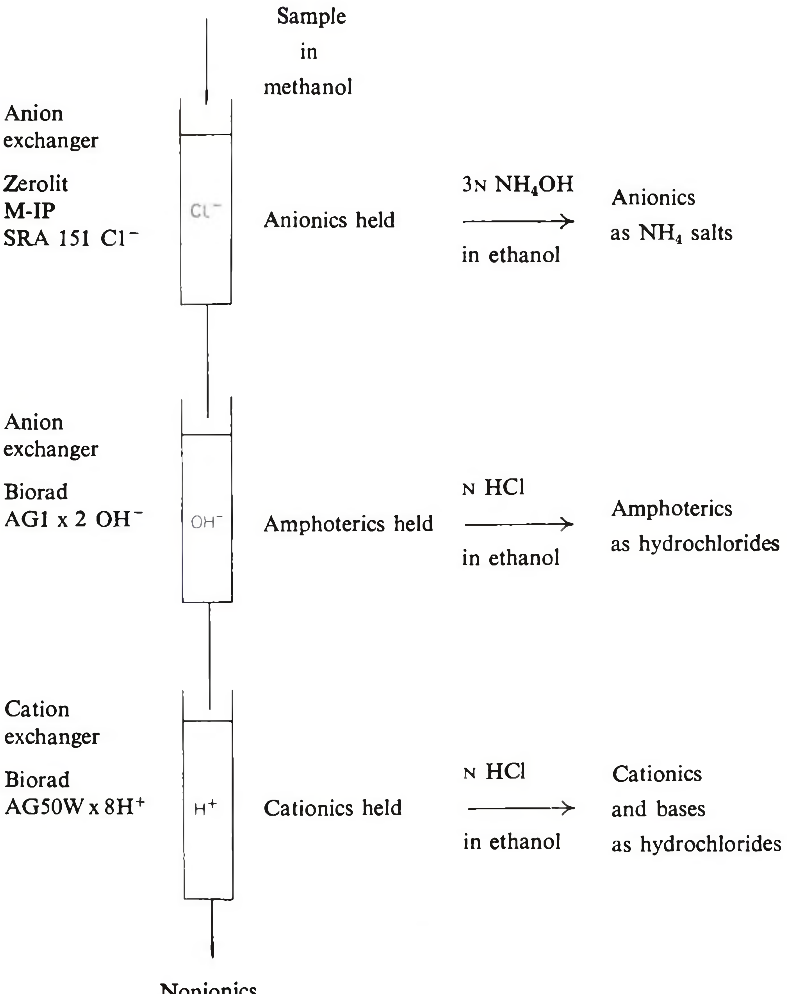

Figure 1. Ion-exchange scheme for separation of surfactant types.

characteristic for many surfactant species and can eliminate a lot of unnecessary work. A simple low resolution infra-red spectrophotometer is suitable for this purpose.

The fraction from the first column contains anionic surfactants as the ammonium salts. The anionics most commonly used in shampoos are alkyl sulphates and alkyl ether sulphates, but other types such as acyl taurates, isethionates, sulphated monoglycerides and sulphonates are found.

The alkyl sulphates hydrolyse readily, by refluxing with dilute acid, to the parent alcohols and the isethionates and monoglycerides to the parent

fatty acids. An indication of the identity of the hydrolysates can be obtained from the ir spectrum since these are characteristic for the different chemical classes.

These fatty alcohols and fatty acids are still mixtures and we need yet another separation technique—one which will permit separation and estimation, namely gas chromatography. For example 'lauryl' alcohol can be separated into its component fatty alcohols on a column with *Carbowax 20M* as liquid phase and a flame ionization detector. The main alcohols are C_{12} and C_{14} with minor amounts of C_{10}, C_{16}, C_{18}. The areas under the peaks can be measured manually by triangulation or preferably by some form of mechanical or electronic integration and from this the percentage composition can be calculated.

The ethoxylated alcohols are in themselves not volatile enough to be separated directly so a more volatile derivative is formed, in this case, the trimethyl silyl ethers.

An ethoxylated fatty alcohol with an average of two molecules of ethylene oxide will have components containing up to eight molecules of ethylene oxide. The trimethyl silyl ethers can be separated with methyl phenyl silicone fluid (OV 17)* as liquid phase and a flame ionization detector. It is not unusual to find 30 or more components and manual triangulation of this number of peaks is tedious, so the advantages of an integrator attached to the recorder or linked to the gas chromatograph become clear (see *Fig. 2*).

Fatty acids from the soaps or the isethionates are converted to the methyl esters before chromatography on a *Carbowax 20M* column so that good Gaussian-shaped peaks are obtained.

Sulphonates do not hydrolyse under dilute acid conditions, but they can be hydrolysed to the parent alkenes by heating with strong phosphoric acid, and these can be determined by gas chromatography.

The fraction from the second column will contain amphoterics. These are more difficult to characterize and identify but they will contain nitrogen and can exhibit acidic and basic characteristics depending on the pH. So the addition of an acid or a base can change the charge on the molecule and this can be noted in the infra-red spectrum. Alkyl betaines usually act as cationics under these chromatographic conditions and appear in the fraction from the third column. Imidazolinium compounds tend to be retained on the columns and only minor amounts of impurities or decomposition products are eluted but the presence of these surfactants can be detected by comparing with reference materials subjected to the same treatment.

* Phase Separation Limited.

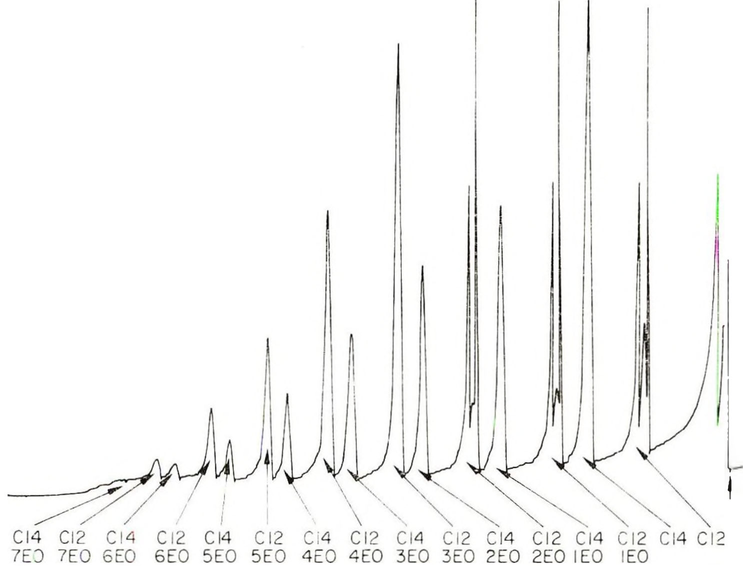

Figure 2. Gas chromatogram of ethoxylated fatty alcohol trimethyl silyl ethers.

The fraction from the third column will contain non-volatile bases as well as cationics. These can be separated and identified by means of two-dimensional thin layer chromatography. The diagram (*Fig. 3*) illustrates the separation of a selection of cationics and weak bases. If it is necessary to know the alkyl chain length distribution in a quaternary ammonium halide, this can be achieved by reaction gas chromatography. In this procedure the first 2 ft of a 5 ft column contains a 20% loading of potassium hydroxide on *Celite* and the last 3 ft contains 10% *Apiezon L* as liquid phase plus 10% potassium hydroxide. The quaternary reacts with alkali on the gc column producing a series of olefins and tertiary amines which can be separated, identified and estimated gas chromatographically (1).

Finally, the nonionic fraction can be separated into its component parts by column chromatography on silica or alumina columns, eluting with successively more polar solvents or preferably by high pressure liquid chromatography which gives better resolution in shorter times. Liquid chromatography is likely to become the generally accepted method for separating surfactant mixtures in the future.

The nonionic fraction is likely to be the most complex since in addition to lather booster (fatty amides), fatty esters for opacifying, and ethoxylated nonionic surfactants, it will contain the nonionic components (e.g. unsulphated fatty alcohols etc.) from the other surfactant species.

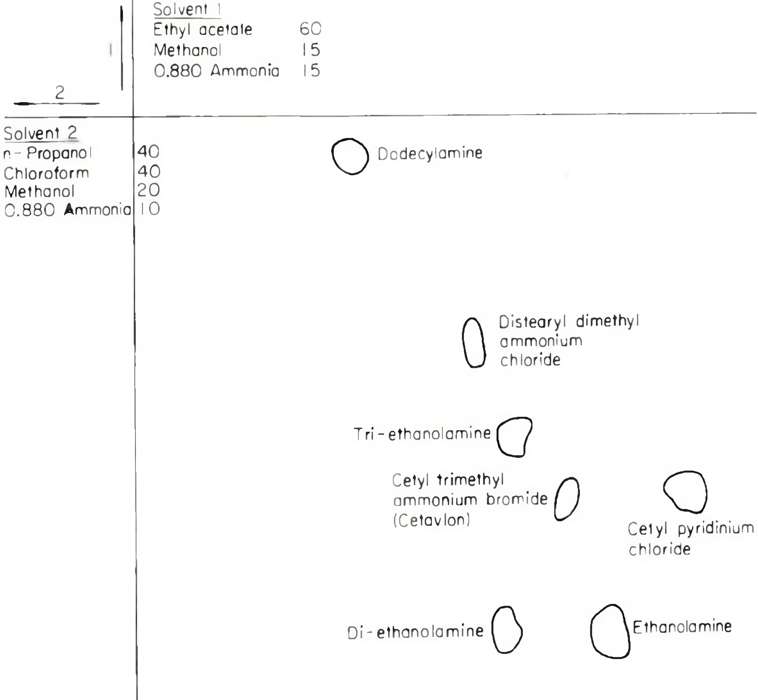

Figure 3. Two-dimensional thin-layer chromatogram of cationics and bases.

Aerosols

Now the hair is clean it needs to be groomed and held in place, so let us consider aerosol hair-sprays and hair dressings.

The components to be analysed consist of propellant, solvent and grooming or setting aid. Propellant/solvent composition is best determined by gas chromatography but the difficulty is the sampling. One of the most successful methods is a can-piercing device (2) which samples from the liquid in the aerosol.

Methanol, ethanol, isopropanol, methylene chloride together with propellants 11, 12 and 114 can be separated on a column containing 25% *Hallcomid M18* (dimethyl stearamide) as liquid phase and a katharometer detector.

The hair setting aid is often a resin or polymer, most frequently synthetic, but natural polymers such as shellac and rosin are used.

An ir spectrum of a film of the polymer is useful for characterization;

this seldom gives enough information for complete identification but a range of other techniques can be used.

The molecular weight distribution of the polymer can be obtained by gel permeation chromatography. This is a form of liquid chromatography and here the detector is a differential refractometer.

High molecular weight species are eluted first followed by decreasingly lower molecular weight substances and finally monomers, inhibitors etc. are eluted. A chromatogram of the non-volatile fraction from an aerosol hairspray can in addition to the polymer give an indication of the presence of other additives.

Glass transition points are characteristic for many polymers and these can be determined by differential thermal analysis (dta). The glass transition point is the stage at which the polymer loses its brittle nature and becomes pliable, but before it actually melts. By determining this point at three different rates of heating and extrapolating back to zero rate a good reproducible determination of the glass transition point can be obtained.

A closely linked technique is thermogravimetric analysis and this can be used to determine the proportions of polyvinyl pyrrolidone (PVP) and polyvinyl acetate (PVA) in the range of PVP/PVA copolymers often used as hair setting aids. There are three temperatures at which a weight loss occurs. The first is at 325°C and this is due to PVA, the second is at 435°C and is due to PVP and the third at 515°C is common to both polymers. By measuring the loss at 325°C compared with the total loss the amount of PVA can be calculated and the loss at 435°C compared with the total loss gives the PVP content.

These results can be confirmed by pyrolysis/gas chromatography. By attaching a Curie point pyrolyser (3) to a gas chromatograph, pyrograms of resins can be obtained and by reference to known polymers the proportions of PVP and VA can be calculated. A pyrolysis temperature of 610°C for 10 s is used and separation is achieved on a column with OVI* as liquid phase and flame ionization detector.

Two hair spray resins which are difficult to distinguish are PVA/crotonic acid copolymer and the terpolymer PVA/crotonic acid/branched chain ester. Both resins could be used in the same factory and it is useful to be able to distinguish them since they have different properties when neutralized in a hair spray. The infra-red spectra are identical but there are subtle differences in the pyrograms. The nuclear magnetic resonance spectra are

* Phase Separation Limited.

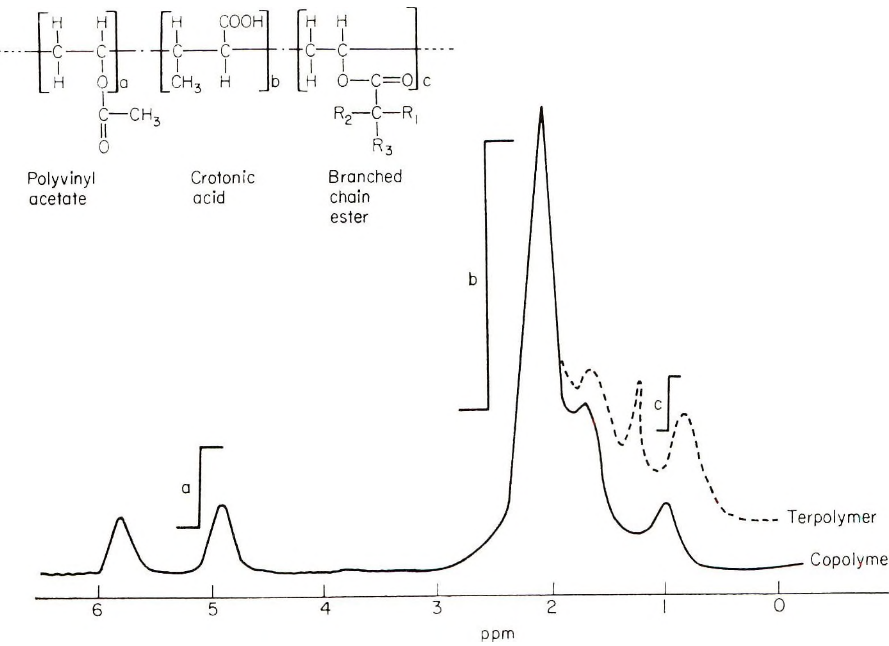

Figure 4. Nmr spectra of polyvinyl acetate/crotonic acid acid copolymer and PVA/crotonic acid/branched chain ester terpolymer.

Table I. Calculated ratios of integrals from nmr spectra for distinguishing between un-neutralized and neutralized PVA/crotonic acid copolymer and PVA/crotonic acid/branched chain ester terpolymer

	(a)/(c)		(b)/(c)	
	PVA/crotonic acid copolymer	PVA/crotonic acid/branched chain ester terpolymer	PVA/crotonic acid copolymer	PVA/crotonic acid/branched chain ester terpolymer
Resin	2.21	0.87	12.78	4.69
Neutralized resin	2.00	0.88	10.22	4.68

also different and the branched chain component of the ter-polymer can be detected easily. The diagram (*Fig. 4*) shows the proton magnetic resonance spectra obtained for the two resins using tetra me hyl silane as internal reference. The integral heights, calculated by electronic integration, are depicted by straight lines above the spectral peaks. The spectral profiles for the two resins are similar except for the region 0.88–1.20 ppm, where the

ter-polymer exhibits an extra peak at 1.20 ppm and a much stronger peak at 0.88 ppm which gives a visual means of differentiating between the two polymers. Confirmation is achieved by comparing the ratio of the integral of peak (a) with that of the methyl peak (c). Further confirmation can be obtained by similarly comparing the integral ratio of peaks (b) and (c) (see *Table I*). At the same time nmr data can give other information, but care must be taken in the interpretation, and confirmation by other means is recommended.

Nmr is also useful for the determination of polyethylene glycol/polypropylene glycol ratios in hair grooming materials. The diagram (*Fig. 5*) shows the proton magnetic resonance spectrum for an ethylene oxide/propylene oxide copolymer with integral heights depicted by straight lines above the spectral peaks, labelled e, p and r. Integral r arises from the CH_3 of propylene oxide and integral e from the two CH_2 groups of ethylene oxide, and the EO/PO mole ratio is calculated from the formula $\frac{3e}{4r}$. If integrals e and p are not resolvable, i.e. for high molecular weight polymers, then the combined integral e + p can be used and the EO/PO mole ratio calculated from the formula

$$\frac{3\,(e+p-r)}{4r}$$

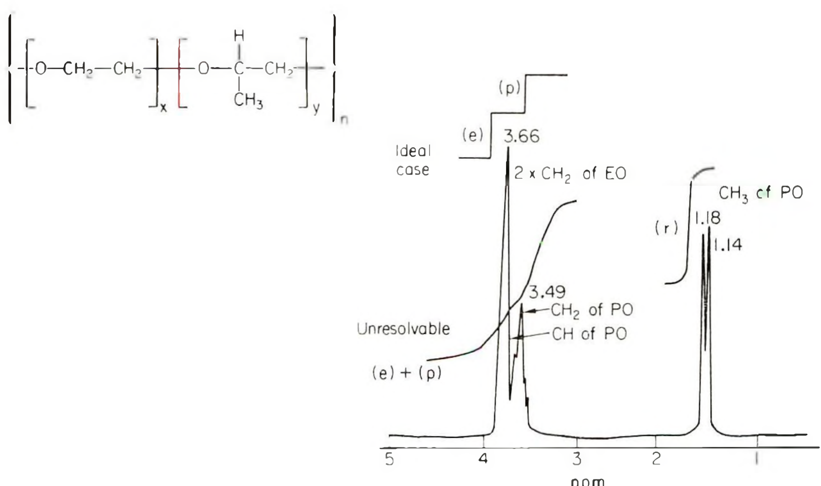

Figure 5. Nmr spectrum of ethylene oxide/propylene oxide type polymers.

The results agree well with those claimed by the manufacturers. Unfortunately, this technique does not differentiate between block, graft, random or alternating polymers.

Toothpastes

There has been considerable research effort in the dental field aimed to prevent, or at least reduce, the incidence of dental caries. Sodium monofluorophosphate is the most common ingredient incorporated in toothpastes for this purpose in the United Kingdom. Since fluoride and other phosphates occur in the commercially available raw material a method is needed which will allow the estimation of fluorophosphate ion.

Monofluorophosphate can be separated from fluoride and other phosphates by means of ion-exchange. Manually this is rather tedious but if sufficient numbers of samples are involved it is convenient to automate the procedure.

The sample is pumped onto the ion-exchange column (anion exchanger Biorad AG 1×8). It is then eluted with an exponentially increasing gradient of potassium chloride solution so that ortho-, monofluoro- and other -phosphates are eluted in this order. The continuous flow of eluent is hydrolysed with strong sulphuric acid, then reacted with ammonium molybdo-vanadate solution to form the yellow complex which is measured colorimetrically, i.e. visible spectrophotometry and recorded. Meanwhile, once the phosphates are eluted the column is regenerated with acid and washed with dilute potassium chloride buffer so that it is ready for the next sample. The peak areas are measured and the amount of monofluorophosphate is calculated by reference to standards passed through the system. It takes 1 h for a complete analysis.

In this system determination of fluoride ion is not included but it is determined in a suitable buffer by means of a fluoride electrode, which is an example of a specific ion electrode. The electrode gives a measure of fluoride ion activity which can be related to concentration.

Determination of sodium and/or potassium in dentifrices can best be achieved by flame emission or alternatively atomic absorption spectrophotometry. Most modern atomic absorption spectrophotometers also have a flame emission facility and whichever method is most suitable for the sample can be used. In atomic absorption mode elements such as calcium, magnesium, aluminium, which often occur in dental preparations,

can be rapidly determined. This technique is suitable for a wide range of metals in most types of toilet preparations and has the advantage over conventional methods in that it is rapid and usually quite sensitive and the sensitivity can be adjusted for high or low concentrations.

Some toothpastes, e.g. in the United States, contain stannous fluoride as the anticaries agent and one may need to know the proportions of stannous and stannic tin in the product.

Atomic absorption does not differentiate between the different states of oxidation but one technique which does is polarography. Stannous tin can be measured in a base electrolyte of hydrochloric acid and ammonium chloride at about -0.5 V and stannic tin in a different base electrolyte—namely ethylene diamine tetra acetic acid di-sodium salt and sodium bromide—measuring the peak at 0.71 V, relative to the mercury pool anode.

Antiperspirants and deodorants

Antiperspirants and deodorants, an area of personal hygiene products, have become much more widely accepted in recent years. Most of these products contain aluminium or zinc salts as the antiperspirant agent together with other materials which act as vehicles to facilitate application, and a deodorant.

After removal of volatiles and chloroform-soluble substances the residual solid substance could contain aluminium chlorhydrate, aluminium chloride, silica, talc, zinc phenolsulphonate etc. Obviously by a series of classical spot tests and estimation of the elements found, the mixture can be analysed.

But if the necessary equipment is available it is possible to complete the whole analysis non-destructively in a few minutes. This involves the use of X-ray microanalysis.

The X-ray microanalyser can be attached to a scanning electron microscope. A small amount of the solid is placed on a graphite block which in turn is mounted in the electron microscope. The X-rays emitted are allowed to build up and a visual display of results is obtained on a cathode ray oscilloscope. By means of a computerized data handling programme quantitative results can be obtained for the elements present. Thus it is possible to ascertain whether there is aluminium chlorhydrate or aluminium chloride or a mixture of both and the proportions of each, whether silica or talc (magnesium silicate) or any zinc salts are present, and to what extent.

This information can be obtained in a matter of about 5 min and in

addition the presence of any other element with an atomic weight greater than that of sodium will be indicated and measured.

This technique of X-ray microanalysis has many applications and can become economically viable if sufficient analyses suitable for this equipment are needed.

The other component in these products is the deodorant and the type of compound used can range from an odour absorber to a germicide. Most of the deodorants can be separated by column chromatography (or liquid chromatography) followed by examination of the uv spectra of the separated fractions. This may not give complete identification since similar classes of compounds give similar spectra. Further separation can be obtained by thin layer chromatography. If these spots cannot be fully characterized then they can be removed, extracted and identified by mass spectrometry. The mass spectrometer is particularly useful for deodorants containing halogens since they produce isotopic clusters of peaks in the molecular ion region and these are readily recognizable (see *Fig. 6*).

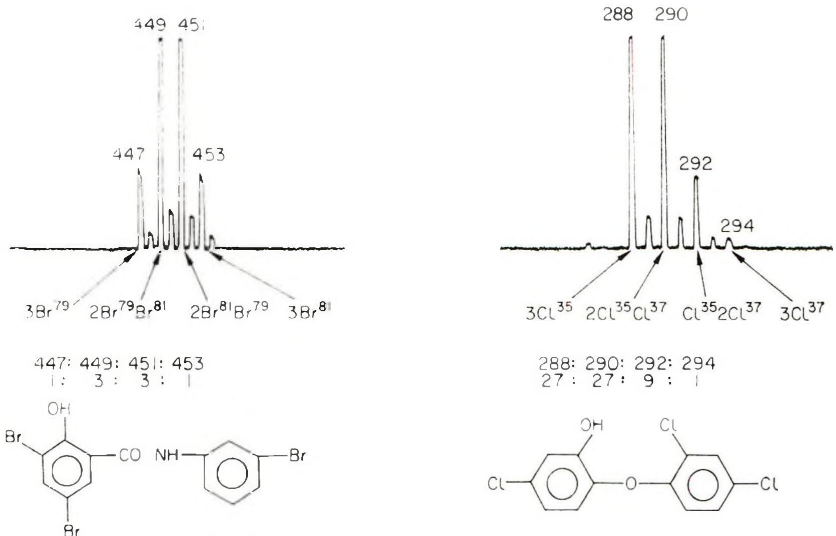

Figure 6. Mass spectra of tribromosalicylanilide (TBS) and Irgasan DP 300.

If other uv absorbing materials interfere it is sometimes possible to use fluorescence spectroscopy. A good example of this is tocopherol, which can be used as a deodorant. The tocopherol is oxidized with nitric acid and reacted with *o*-phenylenediamine to form a phenazine derivative which is fluorescent.

Talcum powder

The safety of products becomes more and more important and currently there is concern about the presence of fibres, which may be asbestos, associated with talc. The FDA in America is concerned about the presence of these fibres in aerosol talcum powders. They have commissioned the analysis of a number of these products by X-ray diffraction. So far no details have been published, but owing to the chemical similarity of talc and asbestos which may be associated with it, X-ray diffraction may be the only way of quantifying the data. By selecting the strongest rings from each material which are not overlapped by rings from the other and measuring each component and known mixtures, the relative amounts in an unknown mixture can be calculated.

Combined techniques

At least one application of each of the techniques listed earlier has been cited in a variety of toilet preparations to give answers to a range of questions which may be asked. For the most part the examples cited have involved a single technique but combined techniques are often used. Pyrolysis/gas chromatography for obtaining pyrograms of resins was mentioned but another useful combination is gas chromatography/mass spectrometry and this combination is very useful for the analysis of flavours and perfumes with gas chromatography giving the separation and estimation and mass spectrometry the identification.

When attempts are being made to identify a complete unknown, a combination of data from a variety of techniques gives the analyst the best chance of success. Ideally, the information should all fit together like a jig-saw puzzle to give one correct unambiguous result. If it does not then somewhere a wrong assumption has been made or data has not been interpreted correctly, and then it is necessary to re-examine all the data and the conclusions drawn from it, possibly supplementing with more data from another technique.

The possibility of future legislation and increased awareness of safety considerations by the consumer may well result in greater demands on the analyst, and this could generate a need to use yet more specialized techniques.

(*Received: 15th March 1973*)

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The application of microcalorimetry to research in the field of toilet preparations

G. P. ADAMS*

Presented at the 7th Congress of the International Federation of Societies of Cosmetic Chemists, in Hamburg, Germany, 19th September 1972

Synopsis—A differential MICROCALORIMETER has been adapted for ADSORPTION studies on biological substrates. The instrument provides reproducible quantitative information on the HEAT change associated with the interaction of the material of interest with the substrate. When this heat change is combined with information on the amount of material adsorbed obtained using conventional analytical techniques, an indication of the adsorbate-adsorbent interaction is obtained. The CALORIMETER is also capable of giving information on the rate of adsorption, the time required to reach thermal equilibrium and whether or not appreciable adsorption occurs in times encountered during product application. Subsidiary experiments indicate whether the adsorbed material is likely to withstand rinsing or whether the adsorption characteristics are grossly affected by the presence of detergent.

The paper describes the apparatus and techniques employed and gives general examples of the applications of the method to illustrate its utility.

Introduction

One of the objectives of a toilet preparations formulator may be to develop a product which deposits a functional ingredient onto a particular surface. This surface could be hair, skin or dental enamel. A feature common to all surface processes is the driving force required to achieve a more stable state by a reduction in the free energy, and it is responsible for the phenomenon known as adsorption.

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For a reaction to go to completion the free energy change, ΔG, accompanying the adsorption must be negative. The well-known thermodynamic equation:

$$\Delta G = \Delta H - T\Delta S$$

expresses the relationship between ΔG, ΔH, the overall heat change accompanying adsorption, and the term $T\Delta S$ where T is the absolute temperature and ΔS the entropy change associated with the reaction.

The process of deposition of a material on a surface is favoured by an increase in entropy, which can be regarded as an increase in the degree of disorder of the system. This increase in entropy will give rise to a favourable contribution to the free energy change associated with the adsorption process. The major entropy changes involved when a material adsorbs on a hydrated substrate are (a) desorption of water (ΔS positive) and (b) adsorption of material (ΔS negative). Whether or not we have an overall favourable entropy change will depend on the relative magnitudes of these two changes.

A calorimeter measures the heat change, ΔH, associated with the reaction under investigation. In the case of adsorption onto a hydrated substrate this is not a simple process. The heat measured may include not only the exothermic heat of adsorption of the species on the substrate, but also any heat associated with the movement of material from a solution environment to the surface of the substrate and an endothermic heat term accompanying any desorption of water. An overall exothermic, that is negative, ΔH is desirable for adsorption, since it is an indication of favourable adsorbate—adsorbent interaction and gives rise to the desired reduction in free energy.

The heat changes which accompany adsorption are often small and it is only with the advent of accurate differential microcalorimetric techniques that it is possible to measure these heat changes and monitor adsorption from solution as it occurs. The present paper describes a differential microcalorimeter that has been adapted for adsorption studies from solution onto biological substrates. Various applications of the method will be outlined illustrating the usefulness of the approach.

THE CALORIMETRIC METHOD

The design of the microcalorimeter is similar to that described by Wadso (1). The twin-cell principle is employed to determine the heat liberated or adsorbed during a reaction, so that all external disturbances are effectively

cancelled. Owing to the high sensitivity of the microcalorimeter, only small amounts of substance are needed for an investigation. Furthermore, it is capable of a wide range of applications since the reactions studied can be momentary or have a duration of several hours. Amounts of heat from $2J$ down to $2 \times 10^{-4}J$ can be measured with accuracy.

A schematic diagram of the calorimeter is given in *Fig. 1*. The cells, one reaction the other reference, are in good thermal contact with the junctions of a large number of thermocouples; the reference junctions are in contact with the surrounding heat sink. Thermocouples for each vessel are connected in series whereas the two thermopiles thus formed are connected in opposition. Temperature differences between the two cells induce a differential voltage signal from the thermopiles, and this is amplified, recorded and integrated.

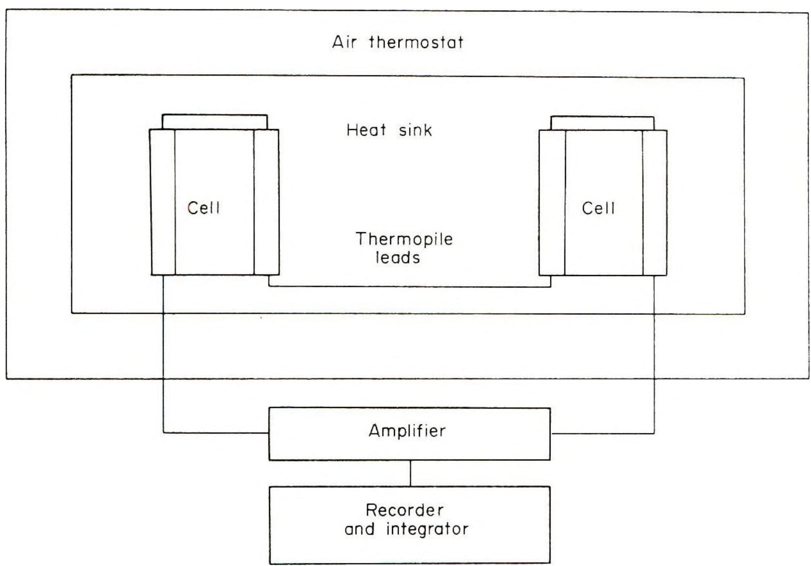

Figure 1. Diagram of calorimeter.

The heat sink, which is rotatable, is positioned in an air bath fitted with a thermostat. The temperature of the bath is regulated by a proportional controller connected to a thermistor in the bath and a heater of resistance wires positioned close to a fan in an outer air container. Cooling water is circulated through a copper spiral also positioned in the outer container. A large perspex box surrounds the air bath, this is maintained at constant

temperature using a 100 W bulb and a mercury contact thermometer fitted in a relay circuit, a small fan is used to move the air in the box. Using this arrangement base-line drift was not observed in the amplification ranges of interest.

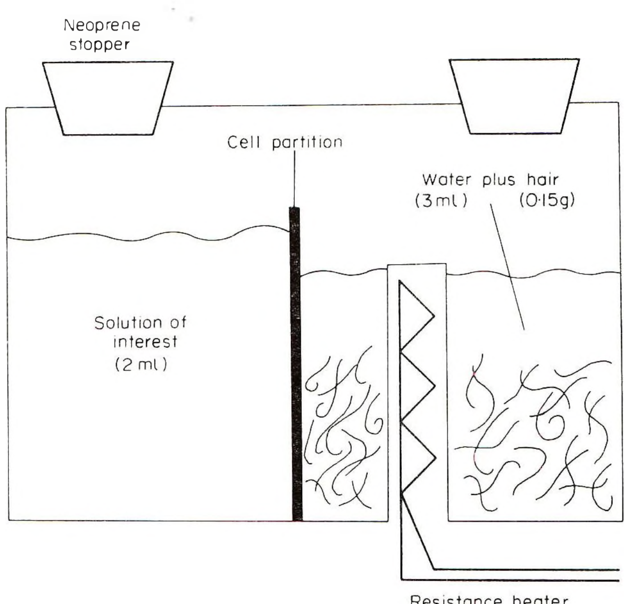

Figure 2. Calorimeter reaction cell.

A diagram of the gold reaction cell is shown in *Fig. 2.* The cell is divided by a partition, which separates the reactants before the reaction. In this case the substrate of interest is hair which is in contact with a known volume of water and the solution under investigation is in the other compartment of the cell. The reference cell contains exactly the same volumes of solution and water as the reaction cell but no hair, so that any heats of dilution are cancelled and the only heat changes measured are those which occur at the solution—hair interface. In order to start the reaction the heat sink is rotated and the reactants mix over the top of the partition. The rotation pattern is such that reactants end up in the large compartment of the cell in contact with the hair. Any temperature change accompanying adsorption on the hair induces a voltage difference in the thermopiles which is recorded as a function of time.

The cells are fitted with 50 Ω calibration heaters. In a calibration experiment a known current, I, is passed through a heater for a known time, t, and the energy, Q_J supplied to the cell is given by the relationship:

$$Q_J = I^2.t.50.$$

The area under the calibration peak, obtained from the integrator trace, is produced by this amount of energy; it is therefore possible to relate the area under the reaction peak to the heat change accompanying the reaction.

In some cases it is desirable to correlate this heat change with the amount of material adsorbed. This gives more information on the magnitude of the interaction between the hair and the adsorbed material and provides some insight into the mechanism of adsorption. If this information is required it is necessary to determine how much material is adsorbed on the substrate using conventional analytical techniques.

Applications of the method

The microcalorimeter which has been described has several applications. It is of use to the background scientist who is interested in the physical factors which influence adsorption on various substrates. Background work carried out in the calorimeter will often give rise to information which can be exploited by the product scientist, who is looking for a functional ingredient which will adsorb substantively from a particular product. The apparatus also provides a quick and convenient indication as to whether a specific compound interacts with a substrate, a use which is appreciated by the product scientist. General applications of the microcalorimeter in background and product research will be discussed.

Background applications

Before it is possible to be more selective in the choice of product ingredients which are likely to adsorb on a substrate, it is necessary to have as much information as possible concerning the factors which influence adsorption. The chemical nature of a compound is of extreme importance in any consideration of its capability of adsorbing on a substrate from solution. This can be illustrated by considering a series of compounds related to phenol, a material which has been shown to adsorb on keratin using conventional analytical techniques (2, 3, 4). The series of interest is cyclohexanol, benzyl alcohol and phenol; the nature of the functional —OH group in

cyclohexanol has been modified in benzyl alcohol, and to a greater extent in phenol, by the presence of an aromatic ring. The heats of adsorption of these three compounds on hair were determined in the calorimeter. The amount of material adsorbed was determined analytically by following the depletion of material in solution after being in contact with hair. Ultra-violet spectrophotometric techniques were employed for the adsorption of benzyl alcohol and phenol, whilst the amount of cyclohexanol adsorbed was determined colorimetrically using the method suggested by Nogare and Mitchell (5). All adsorptions were from 40 mmol solutions at pH 6 and 30°C. The results obtained are shown in *Table I*.

Table I

Material studied	Amount adsorbed ($mmol\ g^{-1}$ of dry hair)	ΔH ($kJmol^{-1}$ adsorbed)
Cyclohexanol	65×10^{-3}	+7·1
Benzyl alcohol (CH_2OH)	110×10^{-3}	−3·8
Phenol (OH)	220×10^{-3}	−6·7

Obviously changes in chemical structure have influenced the adsorption characteristics of these compounds. It can be seen that phenol adsorbs to a greater extent than the other two and the accompanying heat of adsorption is more favourable. Benzyl alcohol adsorbs to a lesser degree with a less exothermic heat change, cyclohexanol still adsorbs on hair but the reaction is endothermic and therefore entropy-controlled.

These observations indicate the change in the adsorption characteristics when the acidic nature of the functional group is influenced by the proximity of an aromatic ring. The implications of the findings are that phenolic-type

materials would be better candidates for substantive adsorption on hair than materials like cyclohexanol which have less acidic —OH groups.

It is this sort of controlled experiment which enables the background scientist to build up rules which govern adsorption on various substrates. The information obtained helps him to suggest materials which are likely to adsorb and give a positive rationale for the choice of product ingredients.

Product applications

The applications of the calorimetric method within product development can be understood by once again considering the adsorption of phenol on hair. *Fig. 3* shows typical heat profiles obtained in a calorimeter experiment; trace A is the electrical calibration whilst trace B monitors the adsorption of phenol from a 40 mmol solution onto hair. Important information can be obtained from a qualitative observation of the trace.

(1) Phenol is adsorbing on hair in an exothermic process.

(2) There is no induction period to the adsorption.

(3) The majority of the heat of adsorption is liberated in the first few minutes of the reaction. This implies that significant adsorption would occur in times normally encountered in product application.

(4) The exothermic reaction is complete in approximately 90 min.

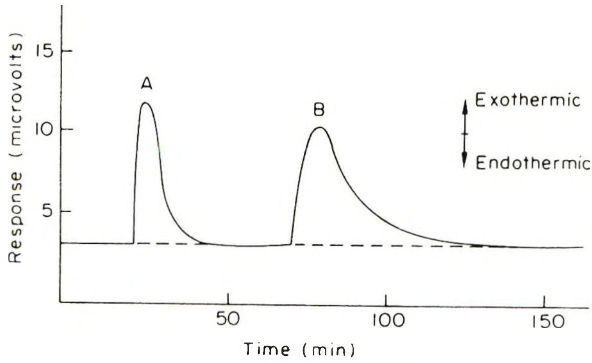

Figure 3. Typical heat profiles.

It is often necessary to know if an additive adsorbs onto a substrate in the presence of another material, such as a detergent. This information can be obtained by slightly adapting the calorimetric method. It is first necessary to study the adsorption characteristics of the materials individually. For example if we were interested in the adsorption of phenol from a 40 mmol

solution containing 5 mmol sodium dodecyl sulphate (SDS) it would be necessary to obtain heat profiles for 40 mmol phenol and for 5 mmol SDS. These are shown in *Fig. 4*; peak A is the phenol profile, whilst B was obtained when 5 mmol SDS adsorbed on hair and consists of a small exotherm followed by an endotherm which continues for many hours before equilibrium is established. It is then necessary to study the interaction of a 40 mmol phenol/5 mmol SDS mixture with hair and obtain a reaction profile. Such a profile is shown in *Fig. 4* peak C. It can be seen that this peak is similar to a combination of profiles A and B. The conclusion can be reached that phenol adsorbs on hair not only from aqueous solution but also from 5 mmol SDS solution. Studies of adsorption from multicomponent systems will mostly be of qualitative use, since it is not known how much interactions between different components will affect adsorption characteristics. It is also important to realize that any impurity present may also have an interaction with the substrate and so it is necessary in all calorimetry studies to deal with well-defined solutions with minimum impurity levels.

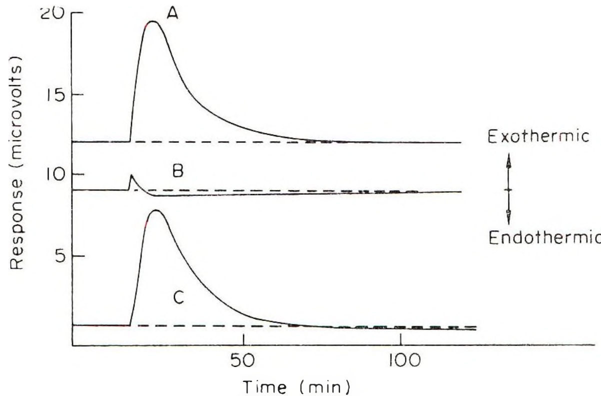

Figure 4. Adsorption in the presence of detergent.

It is of interest to the formulator to know if the adsorbed material is substantive to rinsing. Subsidiary experiments, in which the solutions in the calorimeter cells are progressively diluted, will tell him if this is the case. If desorption does occur a peak of opposite sign to the adsorption peak is obtained. *Fig. 5* shows the profile obtained when the phenol solution from the adsorption experiment is diluted by a factor of three. The endothermic nature of the peak shows that the phenol is desorbing from the hair when the surrounding solution is diluted and is thus non-substantive. Further background work on phenolic compounds has shown that substantivity to

rinsing is achieved by increasing the points of attachment between the adsorbed material and the corresponding binding sites in hair. This is the case with tannic acid (molecular weight approximately 1700), a material which contains a large number of phenol groups.

Tannic acid adsorbs on hair from a 40 mmol solution at pH 6, 14×10^{-3} mmol of tannic acid adsorb per gram of dry hair and each mole of tannic acid adsorbs with an exothermic heat change of -170 kJ mol^{-1}. The adsorption profile is shown in *Fig. 6*; the exothermic peak monitors the adsorption. It can be seen that no peak is obtained when dilution experiments are carried out, indicating that tannic acid is substantive.

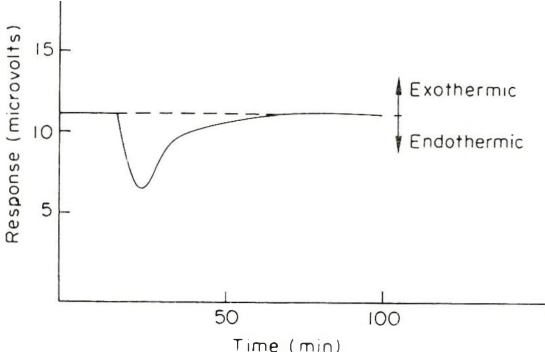

Figure 5. Heat profile from substantivity test.

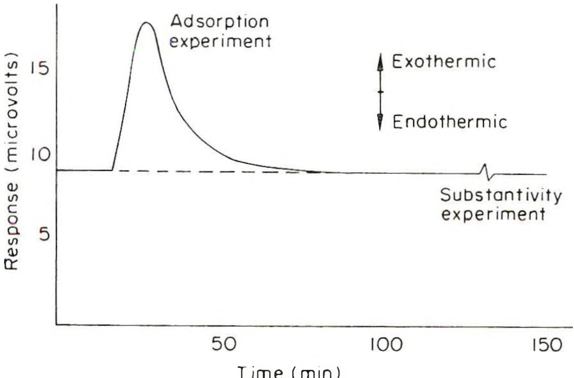

Figure 6. Tannic acid adsorption and substantivity.

CONCLUSIONS

The calorimetric technique has been described and some of the uses have been outlined. In conclusion it is relevant to summarize briefly the main applications of the approach.

The calorimeter indicates in one simple experiment if a material adsorbs from aqueous solution onto the substrate of interest. The kinetics of adsorption and the time required to reach thermal equilibrium are also available.

A subsidiary experiment will indicate if the adsorbed material is removed in the rinsing process.

The calorimetric method can be adapted for studies from solutions containing two additives but the information obtained will mainly be of qualitative use.

When the calorimeter results are combined with analytical data, information on the magnitude of the adsorbate—adsorbent interaction can be calculated.

More detailed background studies on the factors which influence adsorption give information which leads to a more selective approach to the choice of ingredients to be incorporated into products.

(*Received: 23rd July 1972*)

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Society of Cosmetic Chemists of Great Britain Annual General Meeting

The Annual General Meeting of the Society of Cosmetic Chemists of Great Britain was held at the Washington Hotel, London, W.1. on Thursday, 24th May, 1973. The occasion was marked by the presentation of Certificates of Honorary Membership and the award of the 1973 Publications Prize by the Society's President, Mrs Hilda Butler.

The certificates of Honorary Membership were awarded to Mr R. G. Harry, a founder member and past vice-President of the Society and to Mr A. Herzka, a past President of the Society and a former editor of the Journal of the Society of Cosmetic Chemists, in recognition of their services to the Society.

Left to right—Mrs Hilda Butler, President of the Society of Cosmetic Chemists, Dr Colin Prottey, Mr Peter Hartop and Mr Terence Ferguson of the Unilever Research Laboratory, Colworth/Welwyn.

The Publication Prize, which is annually awarded to the best original paper submitted to the Journal, was presented for the second year running to a group from Unilever. The illuminated scroll and £100 prize were awarded to Dr Colin Prottey, Mr Peter Hartop and Mr Terence Ferguson of the Unilever Research Laboratory, Colworth/Welwyn, for their paper 'The Effects of Soap on Certain Aspects of Skin Biochemistry' which was presented at the Society's Oxford Symposium in April, 1972.

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Sedaplant Richter
Soluvit Richter
St. John's Wort Oil
DAB 6 (Erg.-B.)
Tocopherol Oil
0.4% tocopherol
Vitamin $(A + D_2)$ Concentrate
400 000 I.U.A
+ 40 000 I.U.D_3/g
Vitamin B Complex CLR

Vitamin F 250 000 Sh.L.U./g
Vitamin F Ethyl Ester
80 000 Sh.L.U./g
Vitamin F Glyceryl Ester
65 000 - 75 000 Sh.L.U./g
Vitamin F alcohol-soluble
80 000 Sh.L.U./g
Vitamin F water-soluble
25 000 Sh.L.U./g
Vitamin H'
Vitaplant CLR oil-soluble
Vitaplant CLR water-soluble
Wheat Germ Oil 0.26%
$(\alpha + \beta)$ tocopherol

CHEMISCHES LABORATORIUM
DR. KURT RICHTER GMBH

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it may seem like a drop in the ocean

but it's the bitter truth

The normal use of Bitrex as a denaturant for alcohol in the U.K. is 2 parts per million; (incidentally it is detectable by taste at a dilution of 1 part in 20 million parts of water). Bitrex is odourless, colourless, competitive and readily available. If you are not already using Bitrex for your formulations it may be to your advantage to contact us

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DISTRIBUTOR IN THE USA: J. H. WALKER & CO INC. MOUNT VERNON, NEW YORK

Published on behalf of the Society of Cosmetic Chemists of Great Britain by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL. Printed and bound by Burgess & Son (Abingdon) Ltd, Station Road, Abingdon, Berkshire, England.